Treatment of Homocystinuria due to Cystathionine Beta Synthase Deficiency Including use of low-methionine natural-food diet with supplementary cysteine

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Dedication:

To those sufferers unlucky enough to bear the burden of more suffering than the average.

Acknowledgements:

To my mother for her excellent early education of me.

To my father for some genes, and cross-cultural experiences, and for his share of my food and lodging in early life, and perhaps, after all, some, poorly-manifested, affection and regard.

To the University of Queensland Herston Medical Library Staff, for their competence and helpfulness.

To the scientists whose work I have examined in my work here, for their shoulders to stand on.

To the chaotic and causally predetermined universe for my greater share than average of good luck, the disparity being unfair nevertheless; a disparity that I will try to reduce nonetheless the predetermination, because that will make me feel like my life has ‘meaning’, because if I do not try, then certainly my life will have little if any ‘meaning’, and accordingly I will ultimately feel less satisfied than if I do try.

Contents in Brief

Contents in Detail

Introduction

This book(let) is about the treatment of homocystinuria due to inherited severe deficiency of function of the enzyme Cystathionine Beta Synthase.

It is not about the treatment of homocystinuria due to inherited severe deficiency of function of the enzyme Methylene Tetrahydrofolate Reductase, nor about Cobalamin C disorder (probably currently the most frequently detected cause of elevated blood total homocysteine when screened for), nor about the related Cobalamin D, Cobalamin E, and Cobalamin G disorders.

It is not about addressing mild increases in blood homocysteine due to mild deficiency of function of the enzyme Methylene Tetrahydrofolate Reductase, or due to low or deficient folate or low vitamin B12.

It is not about addressing higher blood homocysteine levels of up to about 100 micromoles per litre due to lower or deficient vitamin B12.

The significance, or lack of significance, of mild increases of blood homocysteine levels from the level usually accepted as desirable (10 micromoles per litre), up to about 20 or so micromoles per litre, such as are exciting the interest of various practitioners giving advice to the numerous people in this category, is dealt with in another book, "Homocysteine and Atherosclerosis - Causal Directions in Homocystinurics, the General Population, and Vegetarians" expected to be also available for purchase and freely from the Multifactor Health Website for those unable to afford such purchase in late 2013 or 2014, parts of which have already been posted on the Multifactor Health website.

Introduction continues next page

This book(let) is intended to serve a range of people, from the general public including cystathionine beta synthase deficient homocystinurics and their families, through to scientists, and health and medical personel involved in treatment(s) of the condition(s).

This book is in six main parts and chapters:

Chapter 1. The advice regarding treatment of homocystinurics after infancy (over 3 years) stated as concisely as possible, which is intended to be understandable by the general public, i.e. homocystinurics and their families.

Chapter 2. The advice regarding treatment of homocystinurics during infancy (under 3 years) stated as concisely as possible, which is intended to be understandable by the general public, i.e. homocystinurics and their families.

Chapter 3. The details of how part of this advice was derived from an analysis of the data of the treatment outcome, in terms of thrombosis (formation of undesirable blot clots inside the body, the usual immediate cause of premature death of sufferers from this disease), of five major hospitals (in Dublin (Ireland), Sydney (Australia), Nijmegen (The Netherlands), Manchester and London (The UK)). This is in a form resembling that required for submission to scientific journals, and includes an Abstract and a Concise Summary. The statistical analytic part will be difficult (though not necessarily impossible) for most members of the general public (and less statistically versed scientists and health and medical personel) to follow, but should at least provide a good portion of the general public a glimpse of the nature of imperfections in work done by even well qualified and otherwise generally expert scientists.

Chapter 4. A table summarising or extracting the most important numerical data and word descriptions from literature on Cystathionine Beta Synthase deficiency Homocystinuria, arranged chronologically.

References: a list of scientific journal articles and reference book chapters read by me in the course of writing this book. This does not include hundreds of related articles read in the course of preparing the larger work on homocysteine noted on the previous page.

Peer and expert review comments and discussions. A list of experts in the treatment of homocystinuria due to cystathionine beta synthase deficiency have been provided with the pre-publication final draft of this book, and invited to comment critically under the conditions that they do so independently of each other, that their full responses will be reproduced in this section exactly as they gave them, and that sufficient time for responses by me, and further responses by them, to the conclusion of discussions through agreement or disagreement, be allowed.

Chapter 1. Concise advice for treatment after infancy (from 3 years on):

1.1 Eat no more than 1 gram of protein per kilogram of body weight per day, choosing low methionine foods, in order to keep methionine (one of protein's amino acids) intake low.

Of the low amount in total to be consumed of the high-protein foods (listed immediately below) include a substantial proportion of Macadamia Nuts (which score 0/5 for (methionine + cysteine) and almonds (which score 3/5 for (methionine + cysteine) to keep methionine intake lower still.

Bear in mind that the high-protein foods are also generally high-calorie foods by weight compared to fruit and vegetables, as fruit and vegetables have more water in them – therefore the weight of fruit and vegetables eaten should far exceed the weight of the high-protein foods eaten.

Weighing of all/most foods need only be done until a good visual approximation of the portions is fixed in the memory, and thereafter need only be done occasionally as a refresher of the memory.

The success of the following of these recommendations needs to be determined by serial blood tests of methionine levels, and of blood/ urine tests of homocysteine/ homocystine, in the same way that these tests are used to determine the success of other recommended treatment combinations.

You need to have some knowledge of food protein levels, at least the following generalizations, of percent by weight as usually purchased in retail shops (the list down to and including legumes is of the high-protein foods, and foods listed after that are of lower protein (per energy) level):

Soy Beans (as for the Other Beans, fairly well dried) 40%, wet (drained) after boiling 11 %

Peanuts (a type of bean/legume) 25%,

Other Beans (the seed inside the pod; includes lentils; fairly well dried) 20-25%, wet (drained) after boiling 5 - 6 %

Sunflower Seeds, Sesame Seeds, Pumpkin Seeds 20-25%,

Undried Meats (mammal, bird or fish) 20%,

Almonds, Cashews, Walnuts 17%,

Hazelnuts 15%,

Pecans 12%,

Eggs 12%,

Grains (and grain products like bread) 10-12%,

Pine Nuts/Seeds 7%, (Continued below)

Rice (a grain) 7%,

Yoghurt 4%,

Milk (of any fat content) 3%,

That legumes are a little low in methionine compared to other amino acids regarding human requirements, and that grains, seeds and nuts are a little low in lysine compared to other amino acids regarding human requirements (so eat more of legumes than of grains, seeds and nuts other than macadamia nuts and almonds),

Root vegetables range from:
Carrots 1% to Sweet Potatoes and Potatoes 2%,
Common Green Leaf and Flower (cauliflower and broccoli) Vegetables 2%, uncommon green leafs sometimes being much higher,

Fruits are generally 1% protein by fresh weight, and are even more useful in the diet of homocystinurics than they are in other people (a healthy diet should be mostly fruit by fresh weight) because fruits are generally at the low end of the range of protein:energy ratio of all food groups, and they have an unusually diverse amino acid complement, allowing the choice of fruits whose protein content is unusually low in methionine (ignoring cysteine, because it is much easier to replace this with a capsule supplement than it would be (impossible) to find enough foods high in cysteine but low in methionine to make up a diet acceptable to homocystinurics from) – such fruits include (1/5 is very low, 5/5 would be high in (methionine + cysteine)):

Pawpaw (methionine + cysteine 1/5)

Apples (methionine + cysteine 2/5),

Pears (methionine + cysteine 2/5),

Mangoes (methionine + cysteine 2/5),

Watermelon (methionine + cysteine 3/5),

Bananas (methionine + cysteine 3/5),

Apricots were given as 1/5, but as peaches which are also members of the stonefruit botanical family, are given as 5/5, the dissimilarity seeming unlikely and therefore suspect, I hesitate to include them here, and the same thing applies for mandarins given as 1/5 and oranges as 4/5, both of the citrus botanical family.

(Continued below)

(References: for food protein contents Insel, Turner Ross (2007), Shils, Shike, Ross, Caballero, Cousins (2006), Whitney, Rolfes (2008); for food methionine and cysteine contents the SelfNutritionData website)

Note that previously the common practice has been more to achieve a low enough methionine in the diet by use of a synthetic formula diet with additional breast milk and/or normal foods added as deemed appropriate.
Not enough consideration has been given to the ability to construct an acceptable, palatable diet that homocystinurics can and will use with little burden, based mostly on generally healthy whole fresh foods.
Linder (1991) provided the following table from Acosta and Elsas (1976) of suggested methionine and protein intakes in homocystinuria, compared with the requirements of a normal child of 10 – 12 yrs age.

Age (years)	Methionine (mg/ kg body/ day)	Protein (g/ kg body/ day)	Energy (kcal/ kg body/ day)
0 – 0.5	42	2.00[b]	120
0.6 – 1.0	20	1.50	110
1 – 3	10 – 23	1.25	1300 (total/ day)
4 – 6	10 – 18	1.00	1800 (total/ day)
7 – 10	10 – 13	1.00	2400 (total/ day)
Normal Child			
10 – 12	22	0.81	1500 – 3000 (total/ day)

[b] More is possible if low-methionine mixtures are consumed. ([a] referred to Vit B6 as trialworthy)

It can be seen that the eating of the foods I have given above, in the amounts given, will reduce the dietary methionine to approximately that level suitable for homocystinurics

1.2 Eat only balanced meals,

in order to provide the best combination of amino acids, fats and carbohydrates; and the best blood levels of these over time.

This is to say that each day's intake, eaten only in 2 or 3 balanced meals, should include a total over the entire day no more than 100 grams (50 g for each of 2 meals, or 33 g for each of 3 meals) for (some mixture of, with little or no dairy) all of the beans, nuts, seeds, meats, eggs, dairy products, grains and grain products (e.g. bread etc) together (all the weights of all of these added together).

– alongside this should be a good-sized pile (300-500 g at each meal) of green-leaf vegetables, with or without additional weights of tomatoes and carrots

– and 1.5 – 3 kg (depending on growth and body size) of (combined total weight) starchy root vegetables (e.g. potatoes and sweet potatoes) (less of) and fruit (more of).

(Reference: Vance (2011) for aspects of human diet in general)

1.3 Take cysteine supplements with all meals,
up to 150 mg of L-cystine (two cysteine molecules joined) per kg of body weight per day (divided up into each of the day's 2 or 3 meals), the amount for the particular patient, to normalise blood levels, being determined through a series of blood tests. Capsules or pills may be preferred. Vitamin B6-nonresponsive homocystinurics will need more cysteine than vitamin B6-responsive homocystinurics, as they are producing less of their own (Mudd, Levy, Kraus 2001).

Because, early studies showed clear benefit from cysteine supplementation prior to the vitamin B6 treatment era (Laster, Mudd, Finkelstein et al 1965; Carson, Dent, Field et al 1965; Brenton, Cusworth, Dent et al 1966; Perry, Dunn, Hansen et al 1966; Komrower, Lambert, Cusworth et al 1966; Perry, Hansen, Love et al 1968),
and because nitrogen (and sulphur) balance studies (Poole, Mudd, Conerly et al 1975) do not address some body proteins needing a higher proportion of cysteine than other body proteins, nor do they address the roles and levels of the sulphate (Nimni, Han, Cordoba 2007), and hydrogen sulphide and other potentially important biochemicals (Predmore, Lefer, Gojon 2012) that eventuate from cysteine metabolism (some of its breakdown products, as well as larger molecules it is made a part of).

Also because glutathione, a smallish molecule made (in the body) from cysteine and two other amino acids, is protective against oxidation (is an antioxidant) and potentially enhances immune function (Nimni, Han, Cordoba 2007).

Also because probably it is not only that excess homocysteine interferes with cysteine-cysteine disulfide bonding within and between proteins (i.e. fibrillin (cysteine-rich)) due to homocysteine bonding with the cysteines (and that more strongly than cysteines bind to each other),
but also because a lack of cysteine probably retards the formation of those cysteine-cysteine double bonds thereby leaving unbonded cysteines freely available for longer periods of time so that homocysteine can increase its bonding with them and thus prevent the formation of those cysteine-cysteine double bonds, cysteine deficiency thus probably being an important factor in the disruption of proper formation of some protein fibers that are important parts of the body structures including the walls of arteries and veins, the fibers that retain the lenses of the eyes in their proper positions, and the fibers that are part of correct bone formation (Hubmacher, Cirulis, Miao et al 2010);

Also because amino acids for protein synthesis are not much stored in the body but require being provided fairly immediately together with each other by the body circulation at the time of protein synthesis;

Also because the present studies of homocystinuria treatment benefit do not yet extend into older ages, where problems that have been only slowly developing (as yet undetected) begin to become ((much) more) apparent.
The fact that ascertainment bias here (generally more severe cases being the ones detected earlier in the history of the treatment of the disease, and therefore constituting disproportionately more of the natural history (untreated) reference group of patients in studies of treatment outcomes) has resulted in overestimation of the benefit of treatment for the real population whole group of CBS--homocystinurics (Skovby, Gaustadnes, Mudd 2010) is not a good argument against better treatment – consider how most other preventable and other diseases do not become clinically highly

pathological or urgent until mid-life and beyond for most cases because they include a large proportion of milder cases that only evolve to serious disease more slowly.

Although cysteine supplementation is particularly important (essential, not optional) for those homocystinurics whose Cystathionine Beta Synthase function is approximately nil and cannot therefore be improved much by vitamin B6 supplementation,

it may also be particularly important for those whose cystathionine beta synthase function has enough residual activity to therefore be able to be significantly improved by vitamin B6 supplementation, but who also take folate and/or vitamin B12 and/or betaine in order to further reduce homocysteine levels, because in this case the further reduced homocysteine levels produce less of a drive through the still debilitated Cystathionine Beta Synthase to produce the resultant cysteine, which is therefore further lowered by the folate and/or vitamin B12 and/or betaine treatment that allows homocysteine to escape down that other pathway (remethylation) instead of being forced to some (already suboptimal) extent down the transsulfuration pathway to give some (already suboptimal) amount of cysteine. This despite reduced binding of cysteine by the lowered homocysteine.

Eat only at mealtimes, so that appropriate cysteine supplementation can be achieved, with good proportions of amino acids being absorbed together.

1.4 Take daily vitamin B6 supplements if the homocystinuric is B6-responsive,

the amount for the particular patient, to best as close to normalise blood levels, being determined through a series of blood and/or urine tests.
Homocystinurics who do not respond to B6 with a useful decrease in homocystine should not take B6 supplements, because of undesirable side-effects.
(Yap 2012; Shils, Shike, Ross et al 2006; Mudd, Levy, Kraus 2001; Mudd, Edwards, Loeb etc 1970)

1.5 Take folate, Vitamin B12 and Betaine supplements as appropriate,

to further reduce homocyst(e)ine levels and (the folate) to compensate for excess use of folate due to excess use of the alternative pathway (remethylation) for metabolizing homocysteine. The amount(s) of the particular nutrient(s) for the particular patient, giving the best trade-off between the beneficial effect of lowering homocysteine levels and the risk of any side-effects, to best on balance normalise blood levels, being determined through a similar sort of series of blood tests as for the vitamin B6 supplementation (Yap 2012; Shils, Shike, Ross et al 2006; Mudd, Levy, Kraus 2001).
It should be noted that in the case of B6-responsive homocystinurics, use of these three nutrient supplements (alone or together) reduces the amount of homocysteine forced by higher-than-normal concentrations to go down the transsulfuration pathway via the impaired but B6-partly-restored Cystathionine Beta Synthase enzyme, which reduces the amount of cysteine resulting from metabolism of homocysteine down that pathway, which may increase the need for, or potential benefit from, additional cysteine supplementation to the diet, despite there being less homocysteine to bind to cysteine.

1.6 Eat a largish green salad with all meals, (as noted above) because this is an important part of a generally healthy diet for virtually all humans. (Vance 2011)

1.7 Derive most calories from fresh low-methionine fruit. (as noted above)

1.8 Don't eat fried, roasted, sautéed, grilled, baked or toasted foods,

because the oxidatively damaged fats in these will probably increase whatever damage is happening to artery and probably also vein walls, predisposing to atherosclerosis, and therefore possibly thrombosis also - there is already some artery wall damage in this disease, and it is best to minimize any further damage that may occur from eating these sorts of foods.

Eat raw, boiled, steamed, poached or stewed foods instead.

High heat in the presence of oxygen (i.e. in the air) damages fats, particularly but not only the otherwise generally healthy unsaturated fats.

1.9 Top up total vitamin C intake to 1 gram per day (Pullin, Bonham, McDowell et al 2002) with a supplement that has other antioxidants and nutrients in conservative (lowish, mild) amounts.
Be conservative and avoid higher doses from supplements until more is known in this field of science.

1.10 Regular contact between patients/doctors can be important for helping patients adhere to recommendations.
Perhaps blood sampling should be scheduled so as to maximize maintenance of doctor-patient contact and patient adherence to recommendations.

Chapter 2. Concise advice for treatment in infancy (up to 3 years):

2.1 Combine some amount of breast milk with low-methionine cysteine-supplemented formula food, the correct proportions for the individual being determined by a series of tests

For Newborns and young infants, even if the mother is known to be homocystinuric herself, it is best if some amount of breast milk can be used (preferably in the same meal) together with some of the synthetic low/nil-methionine, cysteine-supplemented feeds as commonly used (Yap 2013).
This is because the breast milk of the first 10 or more days contains a complex mixture of protein and other substances the benefit of which cannot be sensibly measured as merely its amino acid content because much of this protein is not broken down into amino acids in the stomach and intestines of newborn and very young infants, but is absorbed intact into the infants bloodstream to perform various important functions. This probably also applies to a lesser extent after the first 10 or more days.

It has been noted that 'mature' breastmilk as produced 10 or 14 days or so after birth and on thereafter, as well as being very low in protein relative to energy (because humans are by evolution a very low-protein-requiring animal) compared to milks of other animal species (human milk is only a quarter or so in protein:energy ratio as other known mammals' milks (Oftedal 1981)), and also being low in protein relative to energy compared to to other food groups (roughly equal to fruits) regularly eaten by humans. Also, this breastmilk has been said to be the highest in cystine (free cysteine, not in a protein): methionine (ambiguous as to whether free or in protein) ratio (2:1) of all animal products, resembling therein plant tissues (WHO Bulletin 1989).

In any case, be the mother a homocystinuric or not; if a homocystinuric, be she vitamin B6-responsive or –nonresponsive; the correct mixture of breast milk and the synthetic low-methionine, cysteine-supplemeted formula will require determination through a series of tests – tests of the blood levels of the important biochemicals reacting to variation in the doses or ratios of the different treatments applied (breast milk, special formula foods, and vitamin B6 and/or other nutrients (folate, vitamin B12, betaine) – tests that will roughly determine what are the best doses and ratios of the treatments to proceed with. The tests are necessary because there is no ready mathematical formula to calculate the doses and amounts for a particular homocystinuric patient – though there are in some centres some reasonable starting points from which to begin to test variations of treatment dose and ratios, and in the near future hopefully these will be better agreed by consensus and readily available as such in the literature.

Presently, it seems to me that as good an authoritative opinion as any in the world on the details of these matters (on this page) would be that of Professor Sufin Yap of the UK, a practitioner and teacher of inherited metabolic disease treatment with many years of experience with a patient group that for decades now have been more diagnosed and treated in infancy than any other homocystinuric patient group in the world.

Also there are known psychological benefits from breastfeeding, which are part of breastfeeding's known ability to help infants thrive.

2.2 Later in infancy replace some amount of breast milk and low-methionine cysteine-supplemented formula food with low-methionine whole foods and cysteine supplements, the correct proportions for the individual being determined by a series of tests

However, I suggest that as the infant grows older, there is some scope for replacing the presently used synthetic low-methionine cysteine-supplemented formula by the correct combination of low-methionine whole foods and cysteine supplementation as detailed above but with adjustment for the increased protein requirements of infants in accord with the table reproduced above here in section 1.1, of suggested methionine and protein intakes in homocystinuria by Acosta and Elsas (1976) given by Linden (1991). I recommend that this be done in close consultation with the treatment team, who should make themselves aware of the details I have provided here, and done with the standard series of tests of body methionine and homocysteine levels to determine the success of both the diet specifications and the patient's following of them.

Aside to Chapters 1 and 2: There has been some work done on the effect of mutation topology (a fundamental aspect of enzyme shape, here being changed due to the replacement of one amino acid in the/a protein that makes up/ is part of an enzyme, with a different amino acid) on the folding of the protein and therefore on the enzyme's activity (Kozich, Sokolova, Klatovska et al 2010), and on how this might be changed (restored towards normal) by the addition of naturally occurring or synthetic (bio)chemicals (interestingly betaine, taurine and glycerol, all occurring naturally in human biochemistry, have had potentially valuable activity) (Kopecka, Krijt, Rakova, Kozich 2011).
This has some promise for the future, but work has only been done in in-vitro (in a test-tube or other such significantly artificial situation), leaving the question of whether any beneficial changes in humans will be worth paying the price of whatever undesirable side-effects may occur at whatever stage of the patient's life.

Chapter 3. The details of how part of this advice was derived - from an analysis of the data of treatment(s) outcome(s) of Yap et al (2001/2003), and consideration of relevant metabolism, metabolites, and nutrients

3.1 Introduction

This chapter is in a form resembling that required for submission to scientific journals, and includes a Title, author details, an Abstract, a Concise Summary, an Introduction, a Methods, two Backgrounds dealing with important work by other authors, the Results of my own analysis of data from those works, a Discussion including a hopefully sufficiently comprehensive complement of related nutrients, and an Overall Conclusion. References are given in the one Reference section for the whole book.

Title: Analysis of Yap et al (2001/2003) data suggests treatment improvements for prevention of thrombosis in cystathionine beta synthase deficiency are available via improved cysteine supplementation, diet composition, antioxidant supplementation, and follow-up.

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3.2 Abstract

Aim. To further analyse the data of Yap et al (2001/2003).

Background. Homozygous (or compound heterozygous) Cystathionine-beta-synthase deficiency may result in thrombosis. Treatment has included low-methionine diets, cystine-enriched amino acid supplementation and formula foods, vitamin B6, folic acid, vitamin B12, and betaine.

Methods. Data collated by Yap et al (2001/2003) of the thrombosis outcomes of five major (CBS--)-treating centers: in Dublin, Sydney, Nijmegen, Manchester and London; was statistically compared with outcomes predicted by Mudd et al's (1985) untreated natural history outcomes, and then Dublin with the others, and treatments examined.
Consideration was made of homocysteine, cysteine, cystathionine, sulphate, glutathione, folate, vitamin B12, betaine, vitamin C, platelets, acid-base balance, and the metabolites arising in the Oxidation Transamination Methionine Catabolic (OTAMC) pathway: hydrogen sulphide, methane thiol, 3-methylthiopropionate, formate and formaldehyde.

Results. There were less thromboses outcomes (P<.05) in the treated and followed CBS-- patient groups of each of the five groups, even when considered singly, than that expected in the absence of treatment by reference to the natural history data of Mudd et al (1985).
The thromboses outcome of the Dublin group is better than that of the other four groups, but this does not attain statistical significance at the P<.05 level (P ~ 0.16 with the other four groups combined, or P ranging from 0.14 to 0.23 with the other four groups each taken singly). There are differences in treatment regimens.
The considerations of the other metabolites and nutrients suggested potentially important roles for these molecules, such that treatment that included their normalisation or optimisation would be desirable if it could be had at an acceptable, efficient price, which it potentially can to a substantial extent.

Conclusions. Cysteine supplementation may be of more importance than generally acknowledged, and that maybe as a function of the use of homocysteine-lowering treatment modalities other than VitB6.
Low-methionine, high-fruit and vegetable diets may be of more importance than generally acknowledged, and supplementation with vitamin C and other antioxidants should probably be implemented.
Thoroughness, and psychological competence, with regard to the cultivation of treatment compliance is important.

3.3 Concise summary

Homozygous (or compound heterozygous) Cystathionine-beta-synthase deficiency (CBS--) may result in thrombosis, and its treatment has included low-methionine diets, cystine-enriched amino acid supplementation and formula foods, vitamin B6, folic acid, vitamin B12, and betaine.

The data of Yap et al (2001/2003) was further analysed, showing that the thrombosis outcomes of five major (CBS--)-treating centers: in Dublin, Sydney, Nijmegen, Manchester and London, even when considered singly, are better (P<.05) than that expected in the absence of treatment by reference to the natural history data of Mudd et al (1985).

The thromboses outcome of the Dublin group is better than that of the other four groups, although P ~ 0.16 with the other four groups combined, and there are differences in treatment regimens associated with this, suggesting that cysteine supplementation may be of more importance than generally acknowledged, and that maybe as a function of the use of homocysteine-lowering treatment modalities other than VitB6.
This finding, added to which the consideration of other relevant and potentially relevant metabolites and nutrients, suggested that low-methionine, high-fruit and vegetable diets may be of more importance than generally acknowledged, and supplementation with vitamin C and other antioxidants should probably be implemented.
Futhermore, thoroughness and psychological competence with regard to the reinforcement/cultivation of treatment compliance is important.

Key words: CBS, homocysteine, diet, vitamin C, FMD, cysteine

References to electronic databases: Nil

Abbreviations list:
ADP, adenosine diphosphate;
CBS--, homozygous/ compound heterozygous cystathionine beta synthase deficiency (OMIM #236200);
cbEGF, calcium binding epidermal growth factor like (protein moiety);
ECF extracellular fluid;
ICF intracellular fluid;
MDA, malondialdehyde;
MFS, Marfan Syndrome (OMIM #154700);
OTAMC, oxidative transamination methionine catabolism (pathway);
PAPS, 3-phosphoadenosine 5-phosphosulfate;
VitB6, vitamin B6 (pyridoxal phosphate here);
VitB12, vitamin B12;
UK, United Kingdom;
USA, United States of America;

3.4 Background information on homocystinuria resulting from severe homozygous (or compound heterozygous) Cystathionine-beta-synthase (CBS) (EC 4.2.1.22) deficiency

Homocystinuria resulting from severe homozygous (or compound heterozygous) Cystathionine-beta-synthase (CBS) (EC 4.2.1.22) deficiency (CBS--) (OMIM #236200) results in a range of pathologic outcomes in the absence of treatment, including variably thrombosis (the focus of this (Chapter 3) work); premature atherosclerosis, increased Intima-Media Thickness, reduced Flow-Mediated Dilation of vasculature; various skeletal abnormalities including dolichostenomelia/arachnodactyly ('Marfanoid habitus'), osteoporosis, genu valgum, pectus excavatum; developmental delay/ mental retardation, coordination and pyramidal movement disorders; ocular ectopia lentis and myopia; and fatty liver changes (Mudd et al 1985).

Treatment is generally focused on reducing the homocysteine levels of the patient, with the focus on other altered metabolites having varied according to treatment locale or even individual practitioner, though less so lately, with more internally- homogenous treatments applied in locales (ie nations or major city-serviced areas), which are accordingly better differentiable.
Treatment has included low-methionine diets, cystine-enriched amino acid supplementation and formula foods, vitamin B6 (responsivity to this, the major cofactor of CBS, is the criteria for dichotomising CBS--.), folic acid, vitamin B12, and betaine (Yap 2012; Shils, Shike, Ross et al 2006; Mudd, Levy, Kraus 2001; Yap et al 2001).

The objective of this work is to re-examine the work of Yap et al (2001) dealing with the thrombosis outcomes of five major CBS-- treating centers, in Dublin of Ireland, Sydney of Australia, Nijmegen of The Netherlands, and Manchester and London of the UK, and to further (i.e. statistically) compare them not only with Mudd et al's (1985) untreated natural history outcomes, but with each other, and examine the possible relationship of any differences in thrombosis outcomes to respective treatment regimes.

3.5 Comparison of the treatment outcomes in terms of thrombosis (formation of undesirable blood clots inside the body, the usual immediate cause of premature death of homocystinuric patients), of five major hospitals (in Dublin (Ireland), Sydney (Australia), Nijmegen (The Netherlands), Manchester and London (The UK)) having different mixtures of homocystinuric patient types, and using different combinations of treatments.

3.5.1 Methods:

Outcome data collated by Yap et al (2001) of the thrombosis outcomes of five major (CBS--)-treating centers, in Dublin of Ireland, Sydney of Australia, Nijmegen of The Netherlands, and Manchester and London of the UK, was statistically compared (with an adjustment for proportions of VitB6-respondent/-nonrespondent CBS-- treatment years) with outcomes predicted by Mudd et al (1985)'s untreated natural history outcomes (using **only** that portion of Mudd et al's time-to-thromboembolic event curve that is covered by the ageing period between the average age at commencement of treatment, and the average age at the Yap et al (2001) study present time).

The thrombosis outcome results of Dublin of Ireland are then compared to the thrombosis outcome results of the other four centres combined and then individually.

The basic statistical method used is the method for comparison of proportions in independent samples provided by Snedecor and Cochran (1989), the essential equation being:
z = (p1hat – p2hat)/[sqrt(phat*qhat)(1/n1 + 1/n2)],
where: phat = (n of events1 + n of events2)/(n group1 + n group2),
qhat = 1 – phat, and p for statistical significance is obtained by referring z to the table for the normal distribution of z .

The possible relationship of any differences in thrombosis outcomes to respective treatment regimes is then discussed.

3.5.2 Background information from Mudd et al (1985):

The following is the background information from Mudd et al (1985) necessary for thorough comparative considerations:

Mudd et al (1985), in a seminal paper, mailed a standardized questionnaire to each clinician known from a previous study (cited Mudd et al 1981) to be caring for patients with CBS--. Physicians were asked to complete a questionnaire for each such individual about whom they had appropriate information. Further questions focused upon the factor(s) that led to ascertainment, whether the patient was responsive to VitB6, and upon the presence and age of appearance of major clinical manifestations. A detailed history of therapy was requested, as well as a reproductive history….Additional sources of information were identified by a review of the literature and by contacting centers around the world specializing in diagnosis and management of inborn errors of metabolism, as well as by soliciting physician cooperation by notices in appropriate journals. For some patients on whom recent information could not be obtained, published material only was used, if details were available to prove that they did not

overlap. Data collection occurred during 1982 and early 1983 – duplication was searched for, and redundant information deleted.
For their 1985 survey, updated information was received concerning 532 homocystinuric patients with proven or presumed CBS--. To this group was added material from an additional 97 patients obtained primarily from published reports (cites 37 references), bringing the total to 629 patients.

All patients admitted to the study had been demonstrated to be excreting homocystine in conjunction with either enzyme assay or hypermethioninemia or dislocated optic lenses:

Enzyme Assay:	Yes	No
Ectopia Lentis + hyperMet	147(68%)	274(66%)
Ectopia Lentis only	21(10%)	75(18%)
Hypermethioninemia only	39(18%)	64(16%)
Neither of the above	9(4%)	0(0%)
Total	216(100%)	413(100%)

Where hyperMet = Hypermethioninemia

58 were discovered during screening of newborns, and an additional 88 were discovered by screening all siblings (the balance ascertained on clinical features)….

Of the 629 patients, 307 were females and 321 males….

Of the 629 patients, 231 (37%) were classified as biochemically responsive to VitB6 when not folate depleted; 231 (37%) were classified as nonresponsive to VitB6; 67 (11%) were judged intermediate in response; and 100 (16%) had not been classified. For their subsequent analyses only the 231 VitB6-responsives and the 231 VitB6-nonresponsives were analysed.
Of newborn screening ascertainees, 78% were VitB6-nonresponsive, whilst of untreated nonhypermethioninemic cases 90% were VitB6-responsive.
The clinical features leading to investigation for homocystinuria (n = 472 not ascertained by newborn screening or case sibling screening, irrespective of VitB6-response) were:

Clin Feature	SoleCause	ContribCause	Total
Ectopia Lentis	21%	65%	86%
Mental Retardation	4.0	52	56
Developmental Retardation	1.5	21	22.5
Early Thromboembolism	1.1	15	16
Marfanoid Characteristics	0.9	36	37
Bony Abnormality	0.2	23	23
Seizures	0.2	3	3
Behavioral/Psychiatric	0	2.8	3
Other	0.4	10.6	11

They presented the following time-to-event graph (my projection lines) for the first thromboembolic event in untreated patients, both combining and differentiating the VitB6-responders from the -nonresponders. All three curves have a substantially sigmoidal characteristic, such that under the age of

roughly 10 y, the slope of the curve(s) are very roughly half of the maximum slope, that is reached at very roughly 20 years. By 28 years of age the slope(s) has decreased again. As they note, an idea of the differences between the VitB6-responders and –nonresponders is given by the chances of having had the initial clinically detected event by age 15 y being 12% and 27% respectively:

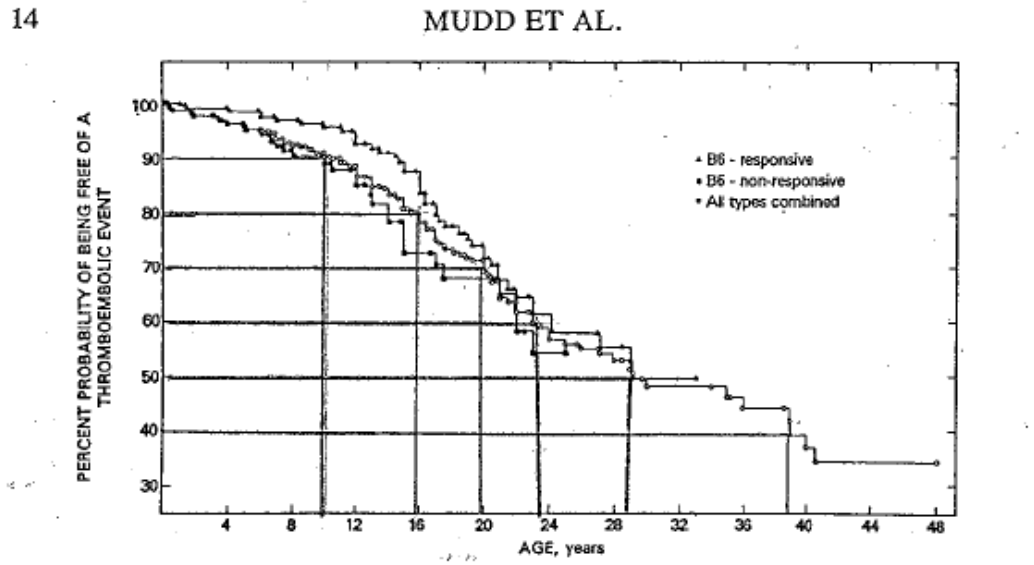

FIG. 4.—Time-to-event graphs for first thromboembolic event in untreated patients. The methods and *symbols* used are the same as those detailed in the legend to figure 3, except that *probabilities on these graphs* are plotted on a linear scale and data for 627 patients were used for *the "all types" curve*. For clarity, *the graph for "all patients"* is plotted starting at approximately age 7.

Figure 1. Reproduction (projections added) of Mudd et al's (1985) Time-to-event graph for first thromboembolic event in untreated cystathionine beta synthase deficiency.

3.5.3 Background information from Yap et al (2001)

The following is the background information from Yap et al (2001) necessary for thorough comparative considerations. Selected data from that they collated, and selected results of theirs relevant to my study here, appear in the table in the Results below:

Now, Yap et al (2001) produced a paper, the abstract for which is reproduced here:

"Abstract…..We performed a multicenter (Dublin, Ireland; Manchester, UK, London, UK, Nijmegen, The Netherlands; Sydney, Australia. DV) observational study to assess the effectiveness of long-term Hcy-lowering treatment in reducing vascular risk in 158 patients.
Vascular outcomes were analysed and effectiveness of treatment in reducing vascular risk was evaluated by comparison of actual to predicted number of vascular events, with the use of historical controls from a landmark study (Mudd et al 1985. DV) of 629 untreated patients with CBS--.
The 158 patients had a mean (range) age of 29.4 (4.5 to 70) years; 57 (36%) were more than 30 years old, and 10 (6%) were older than 50 years.
There were 2,822 patient-years of treatment, with an average of 17.9 years per patient. Plasma Hcy levels were markedly reduced from pre-treatment levels but usually remained moderately elevated.

There were 17 vascular events in 12 patients at a mean (range) age of 42.5 (18 to 67) years:
pulmonary embolism (n = 3),
myocardial infarction (n = 2),
deep venous thrombosis (n = 5),
cerebrovascular accident (n = 3),
transient ischemic attack (n = 1),
sagittal sinus thrombosis (n = 1), and
abdominal aortic aneurysm (n = 2).

Without treatment, 112 vascular events would have been expected, for a relative risk of .09 (95%CI .036 to 0.228; P<.0001)."

Yap (2003) provide additional comment on these findings.

3.5.4 Results (of Vance, here):

Now, firstly, it was not quite proper for Yap et al (2001) to aggregate thromboembolic events in with aneurysms, as they may well have differing metabolite etiologies, and also as diagnosis of aneurysms is not assured.

Secondly, the method of calculation of the number of events expected in the treatment period had treatment not been applied is by no means made clear. The following is an example of what I posit is the most correct method without more complex computational requirements (such as individual data instead of the group averages available from Yap et al 2001) that would probably add little more precision to the outcome; it uses **only** that portion of Mudd et al's curve that is covered by the ageing period between the average age at commencement of treatment, and the average age at the Yap et al study present time:

Taking the average age of 29.4 years, minus the total treatment time divided by n (2822/158 = 17.9 years) one derives that on average treatment commenced at age 29.4y – 17.9y = 11.5y. Extrapolating from the ("All types combined", on the basis that over all five study centres there are very roughly similar numbers of VitB6-responsive and –nonresponsive CBS--) thromboembolic event time-curve of Mudd et al (1985), it can be seen that for an average individual of this group, the probability of having a thromboembolic event, in the absence of treatment, was: 0.52 (the probability of having a thromboembolic event, in the absence of treatment by age 29.4y) **minus** 0.10 (the probability of having a thromboembolic event, in the absence of treatment by age 11.5y) = 0.42.

And, 0.42*158 = 66.4 thromboembolic events expected during the treatment period had treatment not been applied, compared to the 112 given by Yap et al.

Now, take away the 2 abdominal aortic aneurysms, and the 5 repeat thromboses, to leave 10 vascular events instead of the 17 noted (It seems Yap et al did this).

The relative risk would in fact be closer to 0.2 than the .09 claimed.

The .09 relative risk reported is therefore a twofold exaggeration of treatment efficacy. And when it is considered that Mudd's (1985) cases had an ascertainment bias towards more severe cases (in particular out of the vitaminB6-responsive cases), as the index cases (which in the early days constituted a greater proportion of any family case group) in any family group were nearly all discovered clinically, this exaggeration is even more so for the four groups other than Dublin (as they have a lower proportion of vitamin B6-nonrespondent patients than does Dublin), probably nearly a threefold exaggeration of treatment efficacy.
(References related to the ascertainment bias in Mudd's (1985) study being towards more severe cases: Skovby, Gaustadnes, Mudd 2010; Magner, Krupkova, Honzic et al 2011; Kluijtmans, Boers, Kraus et al 1999; Cruysberg, Boers, Trijbels et al 1996; Janosik, Sokolova, Janosikova et al 2009)

More to the point however, is that the treatments applied by these different groups of practitioners are quite different, in particular the Irish compared to the Dutch and to the Australian (See my Table 1 below, and Yap et al 2000), and regarding the usually quite variable cysteine supplementation, which (Yap 2013 personal communication) is virtually universally only applied as part of the proprietary synthetic low-methionine, cysteine-enriched formula food, not as itself, and which has a far from perfect compliance with in that context by homocystinuric patients – usually the later in life treatment commences, the worse the compliance (Wilcken & Wilcken 1997, Walter et al 1998). It seems that with later average age of treatment commencement it is probable it is less attempted to prescribe, and still less achieved of compliance, than in the commendably thorough treatment/monitoring protocols of the Irish group. This lower compliance in the later-diagnosed cases is only somewhat ameliorated in its negative effects, due to the later-diagnosed cases being more often Vit B6-responsive cases, the Vit B6 supplementation being substantially more complied with by homocystinuric patients.
Analysis would more profitably have compared on this basis and sought biochemical theoretical basis for the differences amongst them, rather than proceeding as they have done.
There is also an issue of non-compliant patients being excluded (maybe differentially more so from the Irish study group), but because ultimately further pursuance of this aspect would not change my recommendations, as it very probably does not distort the comparison of treatments (rather, enhances it), maybe only distorting comparison of centers of treatment, I only mention it here for completeness.

I provide in the following table, data reproduced from tables in Yap et al (2001), as well as the results of my own corresponding, and further, analyses (all delineated by "According to Vance"), which includes the appropriate refinement of adjusting the number of thromboses for any compared group by a factor addressing the ratio of Total VitB6-responsive:-nonresponsive treatment years, derived in part from proportionate movement away from the "all types combined" graph line some commensurate distance towards whichever of the VitB6-responsive/-nonresponsive graph lines was appropriate, before projecting across to the y axis.

The fact that the number of VitB6-responsive/-nonresponsive CBS-- are closely proportional to the number of respective treatment years meets one condition necessary for the validity of that procedure.

My tests of statistical significance are by the method for comparison of proportions in independent samples provided by Snedecor and Cochran (1989), done by hand.

The Yap et al (2001) data was drawn by them from: Yap & Naughten (1998), Kluitjmans et al (1999), Wilcken & Wilcken (1997), Yap et al (2000), and Walter et al (1998).

Table 1. Yap et al (2001) data, and comparative and further analyses on it by Vance.

	Dublin	Sydney	Nijmegen	Manchester	London
n CBS--	28	40	30	31	41
n CBS-- followed	27	32	28	30	41
n VitB6-responsive followed	1	17	19	8	25
Total VitB6-resp treat-years	13.7	315	250	183.1	482
AverageVitB6-resp treat-years	13.7	18.5	13.2	22.9	19.5
n VitB6-nonrespons followed	26	15	9	22	16
Total VitB6-nonr treat-years	444.0	288	169	385.5	291
Average VitB6-nonr treat-years	17.1	19.2	18.8	17.5	18.2
Age/y at treatment start	0.8	12.9	22.8	6.7	11.4
Age/y at study present (1998)	18.1	32	38.5	26.5	30.7
n thrombosis events	0	2	1	3	9in4pts
Mudd-predicted n thrombosis According to Yap et al	18	24	16	23	31
P n thrombosis events vs Mudd-predicted n thrombosis According to Yap et al	<.0001	<.0001	.0026	<.0001	.0004
Total treat-years ratio of VitB6-responsive:-nonresp According to Vance	1.00:31.7	1.09:1.00	1.48:1.00	1.00:2.11	1.66:1.00
Mudd-predicted % thrombosis According to Vance	31%	37%	19%	43%	39%
Mudd-predicted n thrombosis According to Vance	8.37	11.8	5.42	12.9	16.1
P n thrombo events vs Mudd-predicted n thrombosis According to Vance	<.0001	.0004	.0414	.0004	.0002
P n thrombo events Dublin vs The other four combined According to Vance	(		0.139a 0.165b		)
P n thrombo events Dublin vs The other four singly According to Vance		0.230b	0.204b	.091a 0.16b	.095a 0.14b

VitB6-responsives					
Free Hcy(ine)	(n = 1) 9.4uM			13.0uM	14.6uM
Total free Hcy		<20uM	7uM		
tHcy (<u>value</u> = derived)	(n = 1)140uM	<u><(40-80)uM</u>	30uM	<u>110uM</u>	<u>110uM</u>
VitB6-nonresponsives					
Free Hcy(ine)	17uM			31uM	33uM
Total free Hcy		33uM	34uM		
tHcy (<u>value</u> = derived)	108uM	<u>80uM</u>	88uM	<u>130uM</u>	<u>130uM</u>
Diet Met/day Restriction (mg/day)	200-625+aaCys	GenAdvice	600	160-900	400-1375
VitB6 (mg/day)	100-800	100-200	200-750	50-500	20-500
Folate	5mg/day	5mg/day	5mg/day	5mg/day	5-10mg/d
VitB12	if deficient	to all	if deficient	nil	50ugoral
Betaine	3-6g/day	6-9g/day	6g/day	4.5-15g/day	2-6g/day
Venupuncture	>=8-10/year	1-4/year	1-2/year	1-4/year	2-4/year

Note: No further detail supplied differentiative of VitB6-responders/–nonresponders.

Where:
a = not adj for Total treat-years ratio of VitB6-responsive:-nonresp
b = adj for Total treat-years ratio of VitB6-responsive:-nonresp
Free Hcy(ine) = plasma free homocystine,
Total free Hcy (fHcy) = free Hcy(ine) + plasma Cys-Hcy,
GenAdvice = general advice
tHcy (<u>value</u> = derived) = signifies that the underlined value has been derived by me from the other values not underlined, presented by Yap et al (2003) but implicitly pertaining to the Yap et al (2001) data. The derivations use the following algorithms:
For Manchester and London a change of the Bonham et al (1997) algorithm of
tHcy = 60uM + 4.5 * (Hcy(ine) <20uM) + 2*(Hcy(ine)>20uM),
to that more consistent with the Given Dublin correlates, that is,
tHcy = 50uM + 3.5 * (Hcy(ine) <20uM) + 2*(Hcy(ine)>20uM);
For Sydney, a rough mean of the tHcy = 40uM + fHcy rule (Wiley et al (1989) with derivations involving the amount of protein per blood volume) and the relationship of the Nijmegen correlates.

So, there is statistically significantly (at the P<.05 level) less thromboses outcomes in the treated and followed CBS-- patient groups of each of the five groups, even when considered singly, to that expected in the absence of treatment by reference to the natural history data of Mudd et al (1985). There is a substantial suggestion that the thromboses outcome of the Dublin group is better than that of the other four groups, but this does not attain statistical significance at the P<.05 level (P ~ 0.16 with the other four groups combined, or P ranging from 0.14 to 0.23 with the other four groups each taken singly).

The possible relationship of any differences in thrombosis outcomes to respective treatment regimes is addressed in the Discussion section below.

3.6 Discussion of results of analysis of Yap et al (2001/ 2003) data by Vance here:

Firstly, my rough derivation, from data provided by Anderson et al's (1991) community-wide study of 16 short-stay hospitals in metropolitan Worcester, Massachusetts, USA, indicates that the population (this would include CBS-- cases) rate of incidence of deep vein thrombosis (DVT) or pulmonary embolism (PE) was one per 20 people over an average lifespan of 80 years. Of course the very large majority of these would occur at ages > 40 years, whereas most of the CBS-- subjects of this work are aged <40 years, and in the four groups other than Dublin had very roughly one deep vein thrombosis or pulmonary embolism per 10 people, at a much younger age than the age at which DVT/PE occurs in the general population.
However, Anderson et al state that because of the silent nature of such events and the low rate of autopsy in the USA, the total incidence, prevalence, and mortality rates of venous thromboembolism remain elusive (and will include the CBS--).
Even given the lack of opportunity for formal statistical comparison of the rates of thrombosis found in the five (four) groups studied here, taking into account the aforementioned aspects, as well as that most of the general population thromboses are preventable by diet and other lifestyle factors like physical exercise and non-smoking, it seems safe to assume that the rates of thromboses observed in the five (the four other than Dublin) groups studied here are substantially in excess of the real rate in the (non-CBS--) general population for these age groups, and very much to the point are very likely to increase with the ageing of the CBS-- patients, and therefore desirable of further lowering.
This latter aspect further validates the carrying out of the numerical and other analyses done here.

Without belabouring the point of the differences in outcomes between Yap et al's (2001) and my analyses (in particular their risk ratio of .09 for treatment versus non-treatment, compared to my calculation (using only the periods of time under treatment, not the period of time from birth, which that difference suggests they used) of the risk ratio being a more modest claim of treatment success of 0.2 (that is, a claim of only half the treatment success) for the five groups combined (and substantially less for the four non-Dublin groups, as noted above)) I move to suggesting that the differences between the Irish outcomes and the other outcomes are worth noting, particularly in the light of small numbers overall plausibly being a factor in the statistical significances of the comparisons not quite reaching any (arbitrary, if generally reasonable) criteria set at $P<.05$.

However, it is necessary to consider the fact that Dublin's (and less so Manchester's) patients were on treatment in early childhood whereas the other centers' patients were not. It is necessary to identify potential biases, their types and their directions, and their potential relative magnitudes and net effect, such as in producing ascertainment bias of cases towards or away from a predisposition to thrombosis.

Note that while on the one hand thromboses are a small part of the symptoms contributing towards ascertainment of the non-newborn-screening CBS-- cases that effectively do not constitute any of the Irish (approx all newborn-screening ascertained) and therefore insofar give an ascertainment bias towards the non-Irish groups having more thromboses, on the other hand the Dublin cases are on CBS genetic bases alone more severe, being of the vitamin B6-nonresponsive type, whereas all the other

centers are much closer (roughly 1:1 to 1:2) to being evenly comprised of B6-nonresponsive and B6-responsive, giving insofar an ascertainment bias towards the Irish group being more predisposed to thrombosis.

It is possible that clinical detection in the non-Dublin cases would drive a higher compliance with treatment, particularly in the earlier-detected vitamin B6-nonresponsive cases, but also in the vitamin B6-responsive cases, due to non-trivial disease manifestations being already experienced by the patient or members of their family, compared to drive from ascertainment via family studies, and even less so for drive from ascertainment by population screening.

Compliance is of course a function of the experienced burden of the treatment, which might be reasonably said to be greater insofar as it included the synthetic low-methionine high-cysteine formula food (though cysteine should be able to be given as a single amino acid in a capsule(s) rather than as a more voluminous mixture of amino acids, relying on food for the other amino acids). However compliance is also a function of patient-doctor contact type and frequency, including the formation of dietary habits very early in life – though peer-group and other societal effects at various other stages of life can override such established dietary habits to varying extent and duration.

The 'total' homocysteine (tHcy) (homocysteine in Hcy-Hcy dimer + Hcy-Cysteine mixed disulfide + Hcy bound to plasma proteins + free homocysteine) levels of the Dublin CBS-- cases are nonetheless in the middle of the range of the five centers (tHcy approximately 110 micromoles per liter versus approximately 80-130), though it might well be the case that the B6-nonresponsive cases in the other centers have higher tHcy, and the B6-responsive cases lower tHcy, than the combined averages given.

Furthermore, the Dublin group of CBS-- experience much more venupuncture in the course of their metabolic monitoring, and venupuncture is noted to promote thrombosis.

Also, the repeat thromboses of the English group have not been incorporated into the analysis, which increases their apparent treatment success in the numerical analysis.

It should be borne in mind that Dublin has absolutely no events of thrombosis (in those on treatment), as yet.

I suggest that a fair adjustment for these differences would put the difference in thromboses outcomes between the Dublin (treated) and the combined other groups in the direction of being more significant, not less significant, and therefore close enough to being statistically significant at the $p<.05$ level to even more definitely warrant further discussion.

Even if earlier treatment of the Dublin group had conferred some advantage other than better later adherence to homocysteine-lowering treatment (this adherence seeming, on the basis of the tHcy levels derived, to be not much better), it is very likely to be due to differences in the body's more permanent structures, rather than to differences in less permanent things like platelets and biochemicals in solution, (though of course these latter might themselves cause differences in the body's more permanent structures). For example the protein fiber scaffolding of tissues including the walls of arteries and veins, in particular the protein fibrillin with its unusually high cysteine content and cysteine-cysteine double bonds.

I take the view that when the bias-relevant factors listed above are roughly though reasonably, and also conservatively, adjusted for, Dublin seems to have better treatment outcomes. I do not claim that this proves that Dublin's treatment practice is better, I claim only that it suggests it.
And consideration also of other metabolites and nutrients as follows here provides a reasonable theoretical support for cysteine supplementation being important.

3.7 Discussion of relevant metabolism, metabolites and nutrients: homocysteine, cysteine, cystathionine; sulphate; glutathione; folate, vitamin B12, betaine; vitamin C; platelets; acid-base balance; and Oxidation Transamination Methionine Catabolic (OTAMC) pathway metabolites hydrogen sulphide, methane thiol, 3-methylthiopropionate, formate and formaldehyde.

3.7.1 Diagram of relevant metabolism:

Consider the following diagram of the metabolic pathways central to considerations of the CBS--situation:

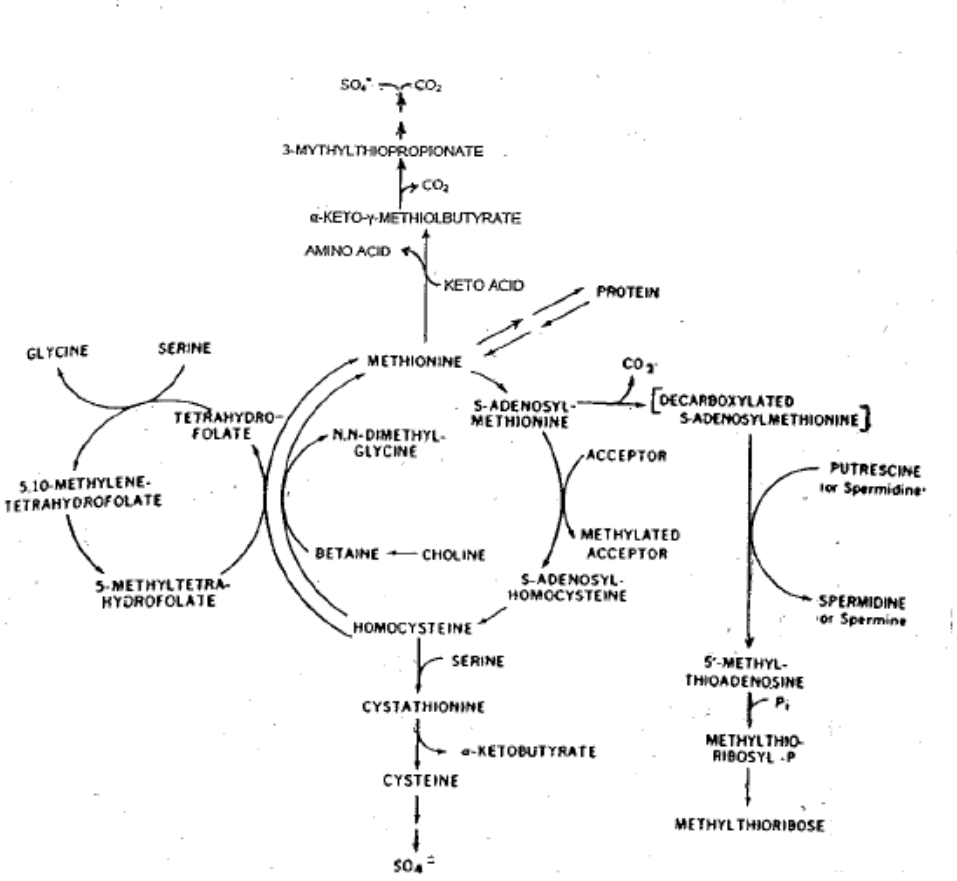

Figure 2. Metabolic pathways central to cystathionine beta synthase deficiency (adapted from Mudd et al's (2001) use of Mudd and Poole's (1975) work)

3.7.2 Sulfate.

"Approximately 19 percent of total sulphate comes from ingested inorganic sulphate from foods and 17% of total comes from inorganic sulphate in drinking water and beverages. Many other sulfur compounds in food can yield inorganic sulphate as a result of degradation or turnover. Among organic compounds, methionine and cysteine in food proteins, glutathione in both animal and vegetable products (Wierbicka et al., 1989), taurine in animal-source foods, lanthionine (a cross-linked sulfur amino acid produced when protein-bound cysteine undergoes heat treatment at an alkaline pH), and sulphated glycosaminoglycans in both plant- and animal-derived foods are important contributors of organic sulphate, providing the remaining approximately 64 percent of total sulphate available for body needs.
…
Table 7.2 shows a fairly similar sulphate content related to calories or good proportion of diet amongst a fairly wide range of foods of different types
…
From Table 7.3 Sulfate levels:
Cow milk 100 mg/ L;
Human milk 5 mg/ L;
Human infant formula 66 mg/ L
…
Human milk is very low in sulfate (5 mg/ L), and even though an average value for infant formula products (both milk- and soy-based) was found to be 13 times higher than that in human milk (66 mg/ L), these levels of sulphate are still lower than those in many sources of drinking water (Hoppe et al., 1998)."
(National Academy of Sciences 2004)

Now, inorganic sulfate is quite readily absorbed from the GIT (Morris and Murer 2001, Florin et al 1991), and this and renal secretion and reabsorption (this latter predominating over secretion, as the molecule is freely filterable; Becker at al (1960)) are quite the main mechanism of sulfate homeostasis. So although, for example, the family of recessively inherited osteochondroplasias, resulting from mutations in the Diastrophic Dysplasia Sulfate Transporter, are due to the undersulfation of proteoglygans in chondrocytes (Satoh et al 1998, Hastbacka et al 1994), it seems unlikely that derangement of sulfation is part of the mechanism of CBS-- pathology, unless in-situ generation of sulfate is a requirement, but this seems unlikely, as there are a number of sulfate-transporters seemingly supplying sulfate from ECF to the ICF of cells (effectively all types of cells?) that require sulfate for the production of 3-phosphoadenosine 5-phosphosulfate (PAPS) for some use(s) or other.

So, given the above information, I will not consider sulfate any further here as an etiologic agent of CBS--, notwithstanding its manifold and varied role in biology, as I have not encountered any information suggesting an important role.

3.7.3 Cysteine.

In accord with the enzyme defect in CBS it has been shown that requirement for cysteine is increased in CBS-- (Laster, Mudd, Finkelstein et al 1965; Carson, Dent, Field et al 1965; Brenton, Cusworth, Dent et al 1966; Perry, Dunn, Hansen et al 1966; Komrower, Lambert, Cusworth et al 1966; Perry, Hansen, Love et al 1968), though even 2% of normal CBS activity may allow the processing of 50% of methionine (via homocysteine) into cysteine (Poole, Mudd, Conerly, Edwards 1975) though the remaining excess methionine/ homocysteine then increases up to high levels, indicating insufficiency. Inspection of the cysteine levels given in Chapter 4 below shows them to be consistently lower than normal, roughly 1/2 to 2/3 of normal, in CBS-- cases considered to be generally satisfactorily treated.

Milewicz et al (2000) review that Marfan Syndrome (MFS, OMIM #154700) is an autosomal dominant disorder affecting multiple systems, including the cardiovascular, ocular and skeletal systems. The cardiovascular pathologies include aortic aneurysms and dissections, and mitral valve regurgitation/ prolapse. Ocular pathologies are ectopia lentis and myopia. Skeletal pathologies include dolichostenomelia, arachnodactyly, kyphoscoliosis, pectus deformities, pes planus, and highly arched palate with crowding of frontal teeth.
They note it has been established that mutations in the gene for fibrillin-1, an elastic fiber, are responsible for most MFS cases.
Sakai et al (1991) report that fibrillin contains 14% cysteine (an unusually high content among proteins), of which one third appeared to be in the free reactive sulfhydryl form. Disulfide bonds play a major role in the fibrillin protein's structure.

CBS-- has in common with MFS many of the abovementioned pathological features (hence the term "Marfanoid habitus of CBS--") (Mudd, Skovby, levy et al 1985; McCully 1969; Perry et al 1968; Brenton et al 1966; Carson et al 1965; Gibson et al 1964), such that in times predating differentiation such CBS-- cases would be lumped in under the MFS diagnosis.

Majors and Pyeritz (2000) report their investigations following on speculation by themselves and others that in CBS-- the altered plasma concentrations of homocysteine and/or cysteine may hinder the synthesis, deposition, or both, of fibrillin-1. They report that when arterial smooth muscle cells were cultured under conditions of cysteine deficiency fibrillin-1 deposition into the extracellular matrix was greatly diminished (and restored with addition of cysteine), but that excessive homocysteine in contrast had little if any effect on fibrillin-1 deposition.
Type I collagen, the major matrix component synthesized by these smooth muscle cells, was not reduced by low cysteine concentrations nor high homocysteine concentrations.

In VitB6-responsive CBS--, the use of VitB6, as a sole treatment, such as to lower methionine to and homocysteine (relatively) near to normal levels, must therefore also have increased cysteine to near normal levels, with regard to its coming from the CBS pathway represented at the bottom of the diagram.
Now, if it is necessary to lower methionine intake to lower methionine and (focally) homocysteine to near normal levels, the question must arise as to whether the products of the CBS pathway, in particular cysteine, become deranged, and to what extent – in this particular case, it would be expected that cysteine would be reduced – less homocysteine to drive the CBS pathway, resulting in less cysteine.

The use of methionine-restriction in non-synthetic food as a sole treatment would be very likely to decrease the supply/ metabolic production of cysteine and to result in cysteine deficiency of some level. Now, if one adds folate, betaine, or VitB12 to the initially (however) successful VitB6-treatment of VitB6-responsive CBS--, this will reduce the drive down the CBS pathway, resulting in less cysteine. At this point it seems reasonable to suggest that a decrease in dietary methionine might benefit from an accompanying addition of cysteine to make good the resultant decrease of the latter.

And likewise, an increase in folate, betaine, and/or VitB12 might benefit from an accompanying addition of cysteine to make good the decrease in metabolic production of cysteine due to the increased folate, betaine and/or VitB12 having routed more of the homocysteine down the remethylation pathway. Use of combinations of these would potentially benefit from further increases in the additional cysteine.

In VitB6-nonresponsive CBS--, methionine-restriction as a sole therapy would be very likely to result in an even more deficient level of cysteine than that pre-treatment, as even careful dietary compositing must reduce cysteine also, as they are substantially positively correlated in foods in both being protein constituents – in practice this compositing constitutes a move to a lower-protein diet, with plant protein sources predominating over animal protein sources.

The addition of folate, VitB12, and betaine to the methionine-restriction and cysteine-supplementation treatment of VitB6-nonresponsive CBS-- would not be expected to result in as lowered, or any at all less, metabolic production of cysteine, as would be the case in VitB6-responsive CBS--.
However, in the event of folate, VitB12 and/or betaine treatments displacing, through either CBS-- patient non-compliance or physician non-emphasis, either the methionine-restriction of diet, or the cysteine supplementation (the latter two both via the same synthetic formula food, in the current standard treatment), then cysteine levels would be reduced accordingly.

Note that the Irish treatment regime is instituted from birth, and is reinforced thereafter in the most thoroughgoing manner of all the CBS-- treatment centres considered here (though the Vit B6-nonresponsivity of their patients is the highest and therefore the most needing of reinforcement), and that it is probable that the Irish accordingly have better compliance with cysteine supplementation than the other centers, particularly if it is over-connected in the minds of the patients/carers with use of the low-methionine (lower-protein) diet. Such over-connection is not valid, particularly in the case of VitB6-nonresponsive CBS--, in particularly the case of non-compliance with any low-methionine diet that has been structured to include higher cysteine:methionine ratio, but also in any case (cysteine-methionine correlation in foodstuffs being sufficiently high as noted above), and it is erroneous and undesirable to view non-compliance with the low-methionine diet as at all a good reason for non-compliance with cysteine supplementation, notwithstanding that cysteine supplementation seems mainly to have been confined to and offered as synthetic low-methionine, high-cysteine formula foods.
In the case of VitB6-responsive CBS-- patients that have been prescribed a low-methionine diet that has not been structured to include higher cysteine: methionine ratio, non-compliance with cysteine supplementation per se (in the apparently unlikely event that it has been attempted to institute supplementation with cysteine as itself) is much less likely to be problematic, but is still undesirable.

Hosoki, Matsuki, Kimura (1997) cite earlier work by one of their group and others (Abe and Kimura 1996) that hydrogen sulfide can be formed from cysteine by (read via) CBS, which is highly expressed in the hippocampus and cerebellum, and that brain homogenates produce hydrogen sulfide, and that

physiological concentrations of hydrogen sulfide facilitate the induction of long-term potentiation in the hippocampus. Relevant to my work here they (1997) present results that they interpret as indicating that endogenous hydrogen sulfide may regulate smooth muscle tone in synergy with nitric oxide.

3.7.4 Other Metabolites: The OTAMC pathway and hydrogen sulfide, methanethiol, 3-methylthiopropionate, formate and formaldehyde.

Discussion of other metabolites involved in CBS-- I am only in a position to deal with as a general concept, but even just the general concept is worth bearing in mind.

In VitB6-responsive CBS--, if *only* VitB6 has been used to successfully lower both methionine and homocysteine to near normal levels, then it may be reasonably said that the resultant levels of *all* metabolites central to CBS-- have been *perhaps close to* effectively normalised – and this situation (*all* metabolites normalised) is probably/possibly the most desirable, practically.

Now, if it is necessary to lower methionine intake to lower methionine and (focally) homocysteine to near normal levels, the question must arise as to whether (other) products of the CBS and oxidative transamination methionine catabolic (OTAMC) pathways become deranged, and if so to what extent.

If one adds folate, betaine, or VitB12 to the initially (however) successful VitB6-treatment of VitB6-responsive CBS--, then this will reduce the drive down the CBS pathway – but, via raised (above threshold >350uM, Tangerman, Wilcken, Levy 2000) methionine, will increase the drive down the OTAMC pathway, producing raised and potentially harmful levels of hydrogen sulfide, methanethiol, 3-methylthiopropionate, formate, and formaldehyde (Blom, Boers, van den Elzen et al 1989, Steele and Benevenga 1979).

In VitB6-nonresponsive CBS--, methionine-restriction as a sole therapy would be very likely to result in an even more deficient level of metabolites downstream of cysteine in the CBS-- than the level pre-treatment, unless careful dietary compositing had not reduced cysteine also (as noted above in the Cysteine section), but in a change towards normal of OTAMC pathway metabolites, particularly if assuming that normally flux through the OTAMC pathway is minimal (Tangerman, Wilcken, Levy 2000).
Addition of sufficient cysteine to methionine-restriction-treatment of VitB6-nonresponsive CBS-- would perhaps seem likely to result in little change in OTAMC pathway metabolites.

The addition of folate, VitB12, and betaine to the methionine-restriction and cysteine-supplementation treatment of VitB6-nonresponsive CBS-- might increase OTAMC pathway metabolites via increased methionine. In the event of these latter treatments displacing, through either CBS-- patient non-compliance or physician non-emphasis, either the methionine-restriction of diet or the cysteine supplementation, then OTAMC pathway metabolites would be expected to increase, or, metabolites downstream of cysteine in the CBS-- to decrease, respectively.

So, there are other metabolites than the ones commonly focused on in CBS--, that are also deranged, and may contribute to some aspect(s) of CBS-- pathology.

3.7.5 Vitamin C.

Pullin, Bonham, McDowell et al (2002) report that after treatment of 5 CBS-- patients (mean age 26 yrs, all treated with vitamin B6, and folate, four treated with betaine, two treated with a "methionine-free protein mix", one treated with VitB12, such as to result in tHcy mean 100 uM vs 5 controls 9 uM) acutely with 2 g of vitamin C once, or chronically with 1 g of vitamin C/ day for 2 weeks or 6 months, endothelium-dependent Flow-Mediated Dilatation (FMD) was restored from a baseline of mean 20um (vs controls mean 116um) to 160um, 170um, and 170um, respectively. This was highly statistically significant (P<.001 for all time-points).
Note that there was no blinding in this experiment, and that CBS-- patient behaviour in other regards, ie compliance to other treatment modalities ie including diet may have changed as a result.

FMD has been found to be strongly negatively associated with the following prothrombotic states:
Systemic hypertension; FMD 4.8% vs controls 8.6%, P<.001 (Felmeden, Spencer, Chung et al 2003),
Stable coronary artery disease; FMD 2.4% vs controls 8.5%, P<.001 (Lee, Blann, Lip 2005),
Primary antiphospholipid syndrome; FMD 8% vs controls 15%, P<.001 (Stalc, Poredos, Peternel et al 2005).
However, it is not clear whether differentiation of the FMD-reduction etiology has a bearing on tendency to thrombosis.
Nevertheless, there is a fairly strong suggestion that reduced FMD might be a contributor to the thromboses found in untreated CBS-- patients, and also to a lesser extent amongst CBS-- patients under some treatment regimes, although of course compliance is always a factor.

Now, 1g of vitamin C would be supplied by (the second digits being almost meaningless in most cases due to the great variability between different batches of fruit or vegetables):
(Fruits)
0.42 kg of Hawaiian guava,
1.4 kg of kiwifruit,
1.7 kg of pawpaw,
2 kg of lychee fruit,
2.3 kg of custard apple,
2.9 kg of rockmelon,
3.6 kg of mango,
5 kg of pineapple,
5.6 kg of honeydew melon or prickly pear,
6.7 kg of bananas,
7 kg of persimmon,
17 kg of apples or grapes,
and not at all by any even vaguely feasible quantity of pears;
("Vegetables")
0.6 kg of raw red capsicums,
0.9 kg of raw broccoli or Brussels sprouts,
1.2 kg of boiled broccoli or Brussels sprouts,
1 kg of raw mustard cabbage or watercress,
1.4 kg of raw red cabbage or raw cauliflower or raw kohlrabi,
2.2 kg of raw common cabbage,

2.8 kg of boiled common cabbage,
3.4 kg of boiled broad beans,
5 kg of boiled potato or sweet potato,
5.3 kg of boiled fresh kidney beans,
5.6 kg of raw common tomato,
6.7 kg of Lebanese cucumber,
8 kg of raw bean sprouts,
8.3 kg of boiled pumpkin,
12 kg of common cucumber,
and not at all by any even vaguely feasible quantity of lettuce and even less so of any legume dried then boiled
(ANZFA 1991).

Furthermore, amongst these and other foods are substantial amounts of other antioxidant nutrients such as vitamin E and carotenoids, which are very likely to reduce the absolute amount of vitamin C required for most of its biological functions, and certainly its antioxidative functions.

It can thus be seen that dietary modifications on their own could probably bring about the vitamin C related increase in FMD noted by Pullen et al (2002). Cultivating complete enough compliance to such dietary modifications might be impossible or too difficult, or inefficient, but even if so, some substantial compliance combined with antioxidant supplementation (vitamin C, vitamin E, carotenoids, and perhaps others) seems to me obviously worth doing, particularly bearing later-life outcomes in mind.

The question then follows as to whether CBS-- treatment regimens that achieve compliance with low-methionine diets, which in practice have/ should have an increased amount of fruit and vegetables, are achieving an improved thrombosis outcome via improved FMD via increased intake of vitamin C and other antioxidant nutrients.

Note that the Irish treatment regime is instituted from birth, and is reinforced thereafter in the most thoroughgoing manner of all the CBS-- treatment centres considered here (though compliance is more crucial for their very predominantly VitB6-nonresponsive patient group), and that it is probable that they accordingly have better compliance (though they have some non-compliance) than those other centers.

Also, it is potentially of importance, in regard to antioxidants, to note that cysteine in the body is joined to glutamate and glycine to make glutathione, which is one of the body's most important antioxidants (see below).

3.7.6 Glutathione

Glutathione is formed in the body from cysteine, joined to glutamate and glycine.

Glutathione is one of the body's most important antioxidants, reducing peroxides (Nimni, Han, Cordoba 2007).

Glutathione also functions normally to keep a substantial portion of the cysteines in protein in a reduced state at their thiol side-chain, rather than those thiols being linked in disulfide bonds to other cysteines in the same protein chain, in different protein chains, or to thiol side-chains of molecules like cysteine and homocysteine.
This activity is probably a function of the extent to which the thiol (the disulphide bond, as acted on) is exposed, and the nature of the other molecule joined to the protein in the disulphide bond.
Note that homocysteine is considered to bond to not only itself, but also to cysteine, more strongly than cysteine bonds to cysteine – this probably applies also in the context of the cysteine being part of a protein chain.

Obviously, then, a shortage of cysteine will potentially, via a shortage of glutathione, have a negative effect both on antioxidation function and on protein assembly and configuration (particularly, on average, the greater the proportion of cysteines there are in a protein, for example fibrillin, a very high-cysteine protein molecule the misfunction of which is very probably involved in the lenticular dysplasia (eye lense displacement) that is characteristic of more severe cases of CBS-- homocystinuria.

3.7.7 Acid-base balance.

Remer (2002) notes that acidification of the human body is more closely tied to higher food sulfur amino acid levels than to food amino acid levels per se.
Based on this and other aspects of his review one would expect the low-methionine diet as commonly employed in CBS-- to lead to a reduction in acidity.
However, the sparse literature (ie Martini, Pusateri, Uscilowicz et al 2005) suggests that acidosis is associated with reduced, not increased, coagulation.
What bearing any alkalizing effect of the low-methionine diet as commonly employed in CBS-- might have on thrombosis (in CBS-- patients) seems unclear.

In a more general sense, it seems that humans generally are evolved to best eat a predominantly vegetarian low-protein diet with enough fruit and vegetables to generally meet most of the water requirement (Vance 2011), and that this would be a more alkalizing diet than the high-protein, low-fruit and -vegetable diets common in most western (and increasingly, unfortunately, in many Eastern) societies.

3.7.8 Cystathionine.

Brenton, Cusworth, Gaull (1965), of autopsies on two CBS-- patients dying of postoperative pulmonary thromboembolism aged 9 years, and 14 controls, reports:
"….the strikingly low concentrations of free cystathionine in all parts of the brains examined in homocystinurics (0.0-0.4 mg/100gWetW) as compared to normal brains from patients dying from entirely unrelated conditions…",
but also
"If (n = 11) the adult control tissues alone are considered, the various areas of brain show striking differences in the concentration of free cystathionine from one area to another: Occipital lobe (12-90) > Frontal lobe (11,13) > Pons and Medulla (8,5) > lateral lobe of Cerebellum (3.5, 2)"
in conjunction with
"The other sulphur containing free amino acids showed no difference either between controls and homocystinurics or between various areas." (This seems suspect, fairly unlikely, to me – the assay techniques, and maybe therefore for cystathinine itself, used here, may have been faulty.)

One meaning of this is basically that cystathionine is potentially not merely an intermediate in the transsulfuration pathway, with no biochemical function of its own, in all tissues. How this might relate to CBS-- pathology is not well defined, even regarding the possibility of involvement in CBS-- patient clumsiness and gait disturbance, where homocysteic acid as a neural excitotoxin must also be considered a possibility (Sommer, Hunzinger, Schillo et al 2004, Adalbert, Engelhardt, Siklos 2002).

Further research is required to elucidate any importance of cystathionine in CBS--.

3.7.9 Homocysteine.

It is beyond the scope of this work to consider the likely involvement of the many postulated mechanisms of homocysteine pathogenesis, with the thrombosis outcome that is the focus of this paper, and this has not been established elsewhere, so I only list the following potential mechanisms noted by recent reviewers:
The high pKa (10.0) of the homocysteine sulfhydryl moiety enabling it to form stable bonds with protein cysteine residues, impairing the function of proteins such as albumin, fibronectin, transthyretin, annexin II, and factor V (Jacobsen, Catanescu, Dibello et al 2005) (Note that the bond strength of homocysteine-homocysteine ("homocystine") is greater than the bond strength of homocysteine-cysteine, which is greater than the bond strength of cysteine-ceysteine ("cysteine"));
angiotoxicity, neurotoxicity, and inhibition of collagen cross-linking (Kuo, Sorond, Chen et al 2005);
impaired vascular endothelium-dependent Flow-Mediated Dilation (Moat and McDowell 2005);
inhibition of endothelial nitric oxide synthase by its endogenous inhibitor asymmetric dimethylarginine, and oxidative inactivation of nitric oxide mediated by upregulation of prooxidant enzymes and downregulation of antioxidant enzymes (Wilson and Lentz 2005);
attenuation of GABA-A/B receptors and increasing redox stress, which activates a disintegrin and metalloproteinase that suppresses tissue inhibitors of metalloproteinase (Tyagi, Lominadze, Roberts 2005).

Also note the report by Hutchinson, Aplin, Webb et al (2004) concluding that reduction of intramolecular calcium binding epidermal growth factor like (cbEGF) domain (i.e. of the cysteine-rich fibrillin-1 elastic fiber protein) disulfide bonds by homocysteine and the resulting disruption of structure may contribute to the change in connective tissue function seen in homocystinuria, but that other cbEGF-containing proteins may also be involved in CBS-- pathology. It seems plausible that both homocysteine excess and cysteine deficiency could play joint roles in at least this latter aspect of CBS-- pathology.

Unfortunately, comparison of the homocysteine levels of the five centers examined in this work provides little information - the algorithms I've used to derive those of the tHcy values that had to be derived, though reasonable, are fairly rough, and the tHcy values have little consistent association with the thromboses outcomes – while the UK centers London and Manchester had both higher tHcy levels and more thromboses than the other three centers, Nijmegen and Sydney had lower homocysteine levels but more thromboses than Dublin.
And here of course the comparison should incorporate VitB6-responsivity, but that differentiation of data is not available from Yap, Boers, Wilcken et al (2001).
Also it is not guaranteed that the different monitoring schedules (in conjunction with other aspects) of the different centers give rise to equal representativity of the assayed homocysteine values of the average homocysteine values, nor that such averages are of more relevance than i.e. maxima.
Any attempt to relate the tHcy values to the treatment regimens should incorporate VitB6-responsivity, but that differentiation of data is not available from Yap, Boers, Wilcken et al (2001), and given the relative closeness of the five regimens, even guarded speculation has little to offer here.
And finally, the tHcy values are not greatly different from each other.

3.7.10 Platelets.

McDonald, Bray, Field et al (1964) used the platelet stickiness method of Wright 1941, McDonald and Edgill 1957, 1958, to report that 5 CBS-- patients aged 4-15y had 46%, 58%, 60%, 48%, 48%, average 54% of platelets remaining at 20 minutes, while the 5 similarly aged controls had 50%, 85%, 77%, 85%, 75%, average 80% of platelets remaining at 20 minutes. However a correction for platelet count (CBS-- average 296,000/ul, control average 216,00/ul) might have decreased the observed difference to little or nothing.

Brenton, Cusworth, Dent et al (1966) report that during the course of nitrogen balance studies in one CBS-- case, SM, "The platelets appeared to be equally abnormal in the first, third and fourth periods and no decreased stickiness had occurred when the last measurement was made 2 days before SM's discharge although the plasma methionine levels had been near normal values for 5 days.
The only change in platelet behaviour was in the second period (supplementary cysteine replaced with methionine) when temporarily they were more sticky than usual and clumped together to such an extent that it was not possible to count them."
They report to the contrary that in trials of a low-methionine, low-protein diet in another CBS--, RM, "Platelet stickiness was measured 3 times in RM. Once before starting the diet, and on the 8th and 22nd day after starting it. On all three occasions the platelets were grossly abnormal in behaviour and the improvements induced by the diet in plasma amino acid concentration (reported: plasma methionine from 12mg/dl to 1-2mg/dl, free homocysteine from 130uM to 100uM) were not obviously associated with any decrease in platelet stickiness….
The dietary trial here was long enough to allow regeneration of all platelets, taking the life of a platelet to be 8 days, so that the failure to improve was not due to the persistence of permanently injured platelets in the circulation."
However, note that the change in homocysteine levels was not great, and not equal to that able to be obtained by full or even substantial compliance with the specified regimens of any of the five centers studied here.

Komrower, Lambert, Cusworth, Westall (1966) report of a trial of a calcium cystinate-supplemented low-protein branched-chain ketoaciduria diet which generally achieved approximately undetectable free homocysteine levels (other than those attending infrequent indiscretions), that is to say tHcy levels approximately <40uM, in CBS-- case treated from infancy to age 2.3 years with good outcome, that "platelet stickiness of 72%" was "considered well within the normal range of this technique."

Harker, Schlichter, Scott et al (1974) reported on the following groups:
Two VitB6-nonresponsive CBS-- patients, diagnosed at age 20 years and 16 years, one of whom had tHcy>200uM and the other probably likewise, had a platelet count of mean 297,000/ul and platelet survival of mean 5.0 days;
Two VitB6-responsive treated CBS-- patients, diagnosed at age 8 years and 16 years, with tHcy of approximately <100uM and 50uM and a platelet count of mean 197,000/ul and platelet survival of mean 8.6 days;
Thirty five normal controls with a platelet count of mean 250,000/ul and a platelet survival of mean 9.5 days.
They carried out further platelet function tests and homocysteine infusion experiments using baboons, to further report that platelet destruction was not due to a direct toxic effect of homocysteine on platelets,

since dipyridamole therapy blocked platelet consumption, nor was it due to enhanced platelet reactivity. They therefore concluded that the underlying process of CBS-- thromboses probably involves formation of platelet thrombus on altered, nonendothelialized endarterial surfaces.

Grobe, Balleisen, Stahl (1979) reported that in three VitB6-responsive CBS-- cases, collagen-, ADP-, and adrenalin-induced platelet aggregation was decreased (not my misprint) before treatment started, and returned to normal when homocystine disappeared from plasma. They concluded that the platelet alterations in untreated patients result from their refractory stage after a release reaction has already taken place, as maybe caused by homocysteine-caused endothelial damage.

Hill-Zobel, Pyeritz, Scheffel et al (1982) harvested platelets from six VitB6-nonresponsive CBS-- patients (treated with VitB6 and folate), six VitB6-responsive CBS-- patients (treated with VitB6), and 11 normal volunteers, labelled them with ^{111}Indium and reinfused them to note no substantial differences (a maximum difference between any two groups of approximately 5% of the survival times found) in platelet survival time according to any of three different mathematical models.

Newman and Mitchell (1984) report on a 20 year-old female CBS-- case diagnosed eventually (and later found to be VitB6-responsive) after thrombotic sequelae following one month after first parturition, whose platelets while on warfarin treatment (no CBS-- treatment) following on serial thrombectomies, had a very high count at 674,000/ul (normal <450,000/ul), and had a stirred, EDTA-added, whole-blood platelet disappearance of 20% in 6 minutes (normal <5%).
However molondialdehyde-generation in clotted whole blood was normal, as was separated platelet rich plasma behaviour with regard to arachidonic acid-induced malondialdehyde production, and response to challenge with ADP, adrenaline and collagen.

In conclusion, it seems reasonable to assume that platelets are more likely to generate thrombi when/after contacting damaged endovascular epithelium, and that reduced FMD causes pressure phenomena that probably increase thrombogenesis (as well as vascular endothelial damage).
These phenomena are likely to apply not only to normal platelets, but also to deranged platelets.
The other obvious inconsistencies in, and incompleteness of, the information discussed on platelet behaviour in CBS-- patients I am unable to resolve in general and in particular with regard to the differences in thromboses outcomes of Dublin versus the other four groups examined here.

3.8 Overall conclusion for Chapter 3.

There seems enough evidence to warrant consideration that cysteine supplementation may be of more importance than generally acknowledged, in the treatment of CBS--, and that it should be seen as a standard part of all treatment regimes, not only as part of a synthetic formula CBS-- diet food.

Perhaps in some cases the cysteine dose should be varied more systematically as a function of the use of homocysteine-lowering treatment modalities other than VitB6 (such as folate and/or vitamin B12 and/or betaine, that reduce metabolic flow of homocysteine down the CBS transsulfuration pathway in VitB6-responsive CBS--, by allowing it to escape down the remethylation pathway), when it becomes potentially even more important, although reduced homocysteine levels resulting in less sequestration of cysteine in homocysteine-cysteine and less other interference with cysteine would (variably) ameliorate this.

Likewise, that low-methionine, high-fruit and vegetable diets may be of more importance than generally acknowledged, and that where compliance with this is compromised (and perhaps in any case, to some extent), supplementation with (at least) vitamin C and other antioxidants should be implemented, though hopefully not in a manner so as to further displace the diet.

Also, the apparent thoroughness of the Dublin group with regard to the reinforcement/cultivation of compliance with dietary treatment, though they do not have a perfect success in this regard, is potentially worthy of study and emulation.
Though, perhaps those patients/ parents who do not comply with low-methionine high-cysteine synthetic formula diets may well potentially comply with an appealing generally healthy low-methionine wholefood high-fruit and vegetable diet with cysteine supplementation via a capsule.

It behoves to consider that these CBS-- cohorts are not yet into older age, when pathologic outcomes in general are greatly increased.

Further study of these matters, at least via ongoing monitoring of treatments/outcomes, and improved detail of data collected, is obviously desirable.

Clarification of platelet behaviour and mechanisms would potentially be helpful.

It is redundant for me to note that further inquiry into the role of homocysteine in disease is desirable, but I add that some more attention to/inclusion of related other metabolites seems warranted.

Chapter 4. A table summarising or extracting the most important numerical data and word descriptions from literature on Cystathionine Beta Synthase deficiency Homocystinuria reviewed by this author (David Vance) in the course of researching this topic, as one topic included in the monograph at present being written on the biomedical significance of homocysteine.

4.1 Chronological References (with key words) for Chapter 4

Notes:
This listing with its abbreviation is most suited for experts in the special field of study; manageable for those with a scientific background; and will be difficult for the general public.
However, all readers including those amongst the general public can benefit from the extraction of the crucial data from the original articles and its collation into Chapter 4 here for readers' convenience – of course the full critical reading of the original journal articles is always the surest way of assessing the meaning of them if one has the background to properly do that.
Some readers may find it satisfactory for their purposes to check if some number of randomly or otherwise selected extractions in the table do indeed fully cover the important information in the original journal article, and if so to accept for their purposes that my extraction of the information from all journal articles has probably been sufficient.

McDonald L, Bray C, Field C, Love F, Davies B (1964) Homocystinuria, thrombosis, and the blood platelets. Lancet, April: 745-746 ; Various hospitals UK, 5CBS-- aged 4-15y, coagulation studies (cites Carson et al 1963). p 54

Gibson JB, Carson NAJ, NeillDW (1964) Pathological findings in homocystinuria. J Clin Path, 17: 427-437 ; Two hospitals, Belfast, Ireland, 7 males and 3 females from 7 families, age 1.5 – 32 years at detection, pathology. p 55

Gerritsen T, Waisman HA (1964) Homocystinuria, an error in the metabolism of methionine. Pediatrics, March: 413-420; Provides the biochemical details for the case RS of Chou SM et al (1965) (see below); U Wisconsin, USA. p 58

Chou SM, Waisman HA (1965) Spongy degeneration of the nervous system: case of homocystinuria. Arch Path,79 (April): 357-363; U Wisconsin, USA 1 case: CBS-- or cbl-- ?, pathology. p 58

Laster L, Mudd SH, Finkelstein JD, Irreverre F, Conerly B (1965) Homocystinuria due to cystathionine synthase deficiency: the metabolism of L-methionine. J Clin Invest, 44(10): 1708-1719; National Institutes of Health, Bethesda, Maryland, USA, 2 related CBS-- and controls (case details in Mudd et al 1964 and Finkelstein et al 1964), methionine metabolism. p 59

Carson NAJ, Dent CE, Field CMB, Gaull GE (1965) Homocystinuria Clinical and pathological review of ten cases. J Pediatrics, March: 565-583; 2 Irish and 1 English hospitals, UK, 10 cases of homocystinuria discovered in a survey of 2,920 mentally retarded individuals in Northern Ireland, clinical, pathological. p 60

Brenton DP, Cusworth DC, Gaull GE (1965a) Biochemical studies of tissues including a comparison with cystathioninuria. Pediatrics (35, January): 50-56; University College Hospital Medical School, London, UK, 2 Hcy-uric children dying at age 9y from pulmonary embolism after eye op, and, 14 controls dying of various other causes, brain regional cystathionine. p 63

Brenton DP, Cusworth DC, Gaull GE (1965b) Homocystinuria: metabolic studies of 3 patients. J Pediatrics, 67(1, July): 59-68; U College Hospital Medical School, London, UK, 2 CBS--, aged 2-5y, 1 CBS-+ Obligate heterozygote parent, 3 control normal adult males, methionine loading. p 64

Schimke RN, McKusick VA, Huang T, Pollack AD (1965) Homocystinuria: studies of 20 families with 38 affected members. JAMA 193(9): 711-719; various eastern USA, mostly examined at Johns Hopkins Hospital, Baltimore, Maryland, USA, 38 CBS-- cases, age 21y (3-45y), roughly normal distributed, skewed a little to the young, clinical, pathological. p 65

Harris ED, Sjoerdsma A (1966) Collagen profile in various clinical conditions. Lancet, Sat 1 Oct: 707-711; National Heart Institute, Bethesda, Maryland USA, 2 mental retarded CBS--, 2 norm IQ CBS--, 34 controls. P 66

Brenton DP, Cusworth DC, Dent CE, Jones EE (1966) Homocystinuria Clinical and dietary studies. Quart J Med, 139, July: 325-349; University College Hospital and Medical School, London, UK, 2 CBS-- cases, dietary methionine and cysteine, nitrogen balance. p 67

Perry TL, Dunn HG, Hansen S, MacDougall L, Warrington PD (1966) Early diagnosis and treatment of homocystinuria. Pediatrics 32: 502-505 ; U British Columbia, Vancouver, Canada, 1 baby boy, CS, 3.5kg birthweight, product of a normal pregnancy and delivery, two elder siblings having previously diagnosed homocystinuria, cow's milk, breast-feeding, low-methionine diet, cysteine.
Note: To compare with 2 other untreated CBS-- siblings, see Perry et al (1968) below. p 69

Komrower GM, Lambert AM, Cusworth DC, Westall RG (1966) Dietary treatment of homocystinuria. Arch Dis Childh 41: 666-671; University College Hospital, London, UK.; 1 CBS-- case with previously diagnosed homocystinuric elder siblings, dietary and plasma cysteine/cysteine. p 70

Gaull GE (1967) The pathogenesis of homocystinuria. Amer J Dis Child, 113, Jan: 103-108; USA, report on a communication received from Perry on the follow-up of the Perry (1966) case (see above), nitrogen balance, cysteine, methionine, taurine, methionine loading. p 70

Perry TL, Hansen S, Love DL, Crawford LE, Tischler B (1968) Treatment of homocystinuria with a low-methionine diet, supplemental cystine, and a methyl donor. Lancet, 31 Aug: 474-478; U British Columbia, Vancouver, Canada, 3 CBS-- cases; methionine, cysteine, pyridoxine. p 71

Carey MC, Donovan DE, Fitzgerald O, McAuley FD (1968) Homocystinuria 1. A clinical and pathological study of nine subjects and six families. Am J Med, July (45): 7-25; 3 Hospitals, Dublin, Ireland, 9 CBS-- cases, pathology reports. p 73

Parkinson MS, Harper JR (1969) Therapeutic problems of adolescent homocystinuria. Proc Roy Soc Med 62(9): 909-910; General Hospital, Northamp-ton, UK?, 1 CBS-- case; dietary noncompliance. p 73

Hopkins I, Townsley RRW (1969) Cerebral thrombosis in a patient with homocystinuria. J Pediatr 75(6/1): 1082-1083; Royal Children's Hospital, Melbourne, Australia, 3 CBS-- sibling cases, thrombosis. p 74

McCully KS (1969) Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis; Am J Pathol 56(1): 111-128; Massachusetts General Hospital, Boston, USA, 1 CBS-- case, pathology. p 74

McCully KS (1970) Importance of homocysteine-induced abnormalities of proteoglycan structure in arteriosclerosis. Am J Pathol 59(1): 181-193; Massachusetts General Hospital, Boston, USA, 2 CBS-- cases, and, 2 control, human cell lines. p 75

McCully KS (1972) Macromolecular basis for homocysteine-induced changes in proteoglycan structure in growth and arteriosclerosis. Am J Pathol 66(1): 83-95 Massachusetts General Hospital, Boston, USA, 2 CBS-- cases, and 2 control human cell lines; sulphate, homocysteine thiolactone. p 75

Cross HE, Jensen AD (1973) Ocular manifestations in the Marfan Syndrome and homocystinuria. Am J Ophth March: 405-420; Johns Hopkins Hospital, Baltimore, Maryland, USA, CBS-- versus Fibrillin gene Marfan Syndrome,
42 CBS-- Homocystinuria, ascertained by amino acid chromatography on ectopia lentis cases referred from ophthalmologists.
142 Marfan Syndrome, all without homocystinuria, ascertained by clinical evaluation, and thereafter unselected. p 79

Harker LA, Slichter SJ, Scott CR, Ross R (1974) Vascular injury and arterial thrombosis. NEJM 291(11): 537-543; U Washington Sch Med, USA, 4 CBS-- cases, 34 controls; Vit B6, pathology, platelet studies. p 80

Poole JR, Mudd SH, Conerly EB, Edwards WA (1975) Homocystinuria due to cystathionine beta-synthase deficiency. Studies of nitrogen balance and sulfur excretion. J Clin Invest 55(5, May): 1033-1048. p 82

Murdoch JC, Rodger JC, Rao SS, Fletcher CD, Dunnigan MG (1977) Down's Syndrome: an atheroma-free model? BMJ 2(6081, July 23): 226-228; Lennox Castle Institution, Glasgow, Scotland, UK, 70 CBS +++ trisomy Downs Syndrome cases versus 70 age- and sex-matched non-Down's mentally defective cases vs 135 normal community controls, blood pressure, atheroma, cholesterol, triglycerides. p 82

Almgren B, Erikson I, Hemmingsson A, Hillerdal G, Larsson E, Aberg H (1978) Abdominal aortic aneurysm in homocystinuria. Acta Chir Scand 144: 545-548; U Hospital, Uppsala, Sweden, 1 CBS-- case, aortic aneurysm. p 83

Grobe H, Balleisen, Stahl K (1979) Platelet function and morphology in homocystinuria. Ped Res 13: 72 ; U Munster, Germany (is an abstract only in the journal), 3 CBS--VitB6-responsives, 5 CBS-- VitB6?non/ responsives already under treatment, platelet studies. p 84

Hill-Zobel RL, PyeritzRE, Scheffel U, Malpica O, Engin S, Camaroo EE et al (1982) Kinetics and distribution of 111-Indium-labelled platelets in patients with homocystinuria. NEJM 307(13): 781-786; Johns Hopkins U, Baltimore, Maryland, USA, 6 CBS--VitB6-responsives, 6 CBS--VitB6-non responsives, 11 normal volunteers, platelet studies. p 84

Wilcken DEL, Wilcken B, DudmanNPB, Tyrrell PA (1983) Homocystinuria – the effects of betaine in the treatment of patients not responsive to pyridoxine. NEJM, 309(8): 448-453; Prince Henry Hospital, Sydney, Australia, 10 CBS--: (by enzyme, Hcy-uria, high methionine), aged 6-37y, (8 VitB6-nonresponsives, 2 VitB6-partially responsives), 25 adult controls, methionine, cysteine. p 85

Dudman NPB, Wilcken DEL (1983) Increased plasma copper in patients with homocystinuria due to cystathionine beta-synthase deficiency. Clinica Chimica Acta 149(2-3): 117-127; Prince Henry Hospital, Sydney, Australia, 14 CBS-- cases (by enzyme, Hcy-uria, high methionine), aged 4-53y, (6 VitB6-nonresponsives, 8 VitB6-(partially?) responsives), and, 44 controls matched for age, sex and smoking, PDO, copper, ceruloplasmin, SOD. p 85

Boers GHJ, Smals AGH, Drayer JIM, Trijbels FJM, Leermakers AI, Kloppenborg (1983) Pyridoxine treatment does not prevent homocystinuria after methionine loading in adult homocystinuria patients. Metabolism, 32(4): 390-397; U Nijmegen, Nijmegan, Netherlands, 8 CBS-- (by enzyme, Hcy-uria, high methionine) aged 20-50y), (mostly non-, with some partially-at-best-, VitB6-responsiveness?..) Vit B6, methionine, cysteine. p 86

Jackson GM, Grisolia JS, Wolf PL, Jones OW, Bloor CM (1984) Postoperative thromboemboli in cystathionine beta-synthase deficiency. Am Heart J 108(3(1)): 627-628; U California, San Diego, USA 1 CBS-- case, Vit B6, coagulation studies. p 87

Newman G, Mitchell JRA (1984) Homocystinuria presenting as multiple arterial occlusions. Quart J Med New Series LIII(210): 251-258; U Hospital, NottinghamUK, 2 CBS—siblings, thrombosis, coagulation studies, cysteine. p 88

Wilcken DEL, Dudman NPB, Tyrrell PA (1985) Homocystinuria due to cystathionine beta-synthase deficiency – the effects of betaine treatment in pyridoxine-responsive patients. Metabolism 34(12): 1115-1121; Prince Henry Hospital, Sydney, Australia, 6 CBS-- (by enzyme, Hcy-uria, high methionine), aged 11-53y, 17 Controls: Normal, aged 21-58y. VitB6-y responsives), Vit B6, folate, betaine, methionine loading, methionine, cysteine. p 90

Mudd SH, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, Andria G, Boers GHJ, Bromberg IL, Cerone R et al (1985) The natural history of homocystinuria due to cystathionine beta-synthase deficiency. Am J Hum Genet 37(31): 1-31; Worldwide survey coordinated from USA; natural history of CBS-- in detail. p 91

Sartorio R, Carrozzo R, Corbo L, Andria G (1986) Protein-bound plasma homocyst(e)ine and identification of heterozygotes for cystathionine-synthase deficiency. J Inherit Metab Dis 9(1): 25-29. U Naples, Naples, Italy; 8 CBS-- cases (by enzyme, Hcy-uria, aged ?), 7 VitB6-responsive, 1 VitB6-nonresponsive, 15 Controls, normal, adults, protein-bound versus free homocysteine. p 96

Abbott MH, Folstein SE, Abbey H, Pyeritz RE (1987) Psychiatric manifestations of homocystinuria due to cystathionine beta-synthase deficiency: prevalence, natural history, and relationship to neurologic impairment and vitamin B6-responsiveness. Am J Med Gen 26: 959-969; Johns Hopkins U, Baltimore, Marylands, USA, 63 CBS-- cases, pathology, psychiatry. p 96

Kempster PA, Brenton DP, Gale AN, Stern GM (1988) Dystonia in homocystinuria. J Neurol Neurosurg Psychiatry 51: 859-862; University College, London, UK, 3 CBS--, psychiatry, pathology. p 97

Wiley VC, Dudman NP, Wilcken DE (1988) Free and protein-bound homocysteine in cystathionine beta-synthase deficiency: interrelations during short- and long-term changes in plasma concentrations. Metabolism 38(8 (August)): 734-9 Prince Henry Hospital, Sydney, New South Wales, Australia, different groups of CBS-- cases on different treatments of Vit B6, folate, betaine; free versus protein-bound cysteine. p 98

Wiley C, Dudman NPB, Wilcken DEL (1989) Free and protein-bound homocysteine and cysteine in cystathionine beta-synthase deficiency: interrelations during short- and long-term changes in plasma concentrations. Metabolism 38(8(August)): 734-739; Prince Henry Hospital, Sydney, New South Wales, Australia; VitB6, folate, betaine; methionine load; cysteine. p 99

Brattstrom L, Isrealsson B, Tengborn L, Hultberg B (1989) Homocysteine, factor VII and antithrombin III in subjects with different gene dosage for cystathionine beta-synthase. J Inher Metab Dis 12(4): 475-482; U Lund U Gothenburg, Sweden, 3 CBS--, 20 CBS-+, 46 normal CBS++, 9 Downs Syndrome CBS+++, coagulation studies, methionine load. p 99

Cochran FB, Sweetman L, Schmidt K, Barsh G, Kraus J, Packman S (1990) Pyridoxine-unresponsive homocystinuria with an unusual clinical course. Am J Med Gen 35: 519-522 ; U California, San Francisco, USA, 1 CBS-- case, pneumothorax, DVT, betaine. p 100

Rubba P, Murcurie M, Faccenda F, Iannuzzi A, Irace C, Strisciuglio P, Gnasso A, Tang R, Andria G, Bond MG, Mancini M (1990) Premature carotid atherosclerosis: does it occur in both familial hypercholesterolemia and homocystinuria? Stroke 25(5): 943-950 ; U Naples, Naples, Italy, 14 CBS-- cases, 14 Controls, 13 CBS-+, 47 Controls ; BP, arterial lesions. p 100

Wagstaff J, Karson M, Kraus JP, Levy HL (1991) Severe folate deficiency and pancytopenia in a nutritionally deprived infant with homocystinuria caused by cystathionine beta-synthase deficiency. J Pediatrics 118(4(1)); Harvard Medical School, Boston, Massachu-setts, USA, 1 CBS-- case, megaloblastosis, pancytopenia, folate. p 101

Monreal M, Callejas JM, Martorell A, Silveira p, Gallego M, Lafoz E, Casals A (1991) Occlusive arterial disease as a form of presentation of homocystinuria. J Cardiovasc Surg 32: 137-138; U Hospital Badalona, Bercelona, Spain; 1 CBS-- case, DVT. p 101

Nordstrom M & Kjellstrom T (1992) Age dependency of cystathionine beta-synthase activity in human fibroblasts in homocyst(e)inemia and atherosclerotic disease. Atherosclerosis 94(2-3): 213-221; U Lund, Malmo, Sweden, 7 CBS--, 14 CBS-+, 20 CBS++ atherosclerotics, 29 CBS++ controls age>=45, 20 CBS++ Controls age<=44, 7 CBS+++ Down's Syndrome, CBS activity. p 101

Mansoor MA, Ueland PM, Aarsland A, Svardal AM (1993) Redox status and protein binding of plasma homocysteine and other aminothiols in patients with homocystinuria. Metabolism 42(11 (Nov)): 1481-5; U Bergen, Bergen, Norway; 7 CBS-- cases, free and protein-bound homocysteine and cysteine. p 102

Celermajer DS, Sorensen K, Ryalis M, Robinson J, Thomas O, Leonard JV, Deanfield JE (1993) Impaired endotelial function occurs in the systemic arteries of children with homozygous homocystinuria but not in their heterozygous parents. JACC 22(3 (Sep)): 854-858; Hospital for Sick Children, London, UK, 9 CBS-- children, age 11+/-1.3y, 33%Male, 18 control children, age 12+/-.7y 33%Male, cholesterol, Vessel Size, FMD, NTG, resting blood flow, reactive hyperemia, IQ. p 102

Allen RH, Stabler SP, Lindenbaum J (1993) Serum betaine, N,N-dimethylglycine and N-methylglycine levels in patients with cobalamin and folate deficiency and related inborn errors of metabolism. Metabolism 42(11 (Nov)): 1448-1460; U Colorado, Denver, Colorado, USA, 5 CBS—cases, betaine, NNDMGly, NMGly, methionine, cystathionine, tCys, betaine. p 103

Rubba P, Mercuri M, Faccenda F, Iannuzzi A, Irace C, Strisciuglio P, Gnasso A, Tang R, Bond MG et al (1994) Premature carotid aterosclerosis: does it occur in both familial hypercholesterolemia and homocystinuria? Ultrasound assessment of arterial intima-media thickness and blood flow velocity. Stroke 25(5 (may)): 943-950 ; U Frederico II, Naples, Italy, 11 normal controls, age 21-35, 12 CBS-- cases, age 8-42y, 10 Familial Hyper-cholesterolemia cases, age 4-49y, wall thicknesses of carotid arteries, flow velocities in middle cerebral arteries, heart rate and pulsatility index. p 104

Schienle HW, Seitz R, Nawroth P, Rohner I, Lerch L, Krumpholz B, Kraus G, Fowler F, Baumgartner R, Willenbockel U et al (1995) Thrombomodulin and ristocetincofactor in homocystinuria: a study in two siblings. Thromb Res 77(1): 79-86; Philipps U Baldingerstr; Marburg, Germany; 2 CBS-- cases, thrombosis, coagulation studies. p 105

Applegarth DA, Vallance HD, Secombe D (1995) Are patients with homocystinuria being missed? Eur J Pediatr 154: 589; U British Columbia, Vancouver, Canada, n = ? CBS-- cases, total versus free homocysteine. p 105

Sebastio G, Sperandeo MP, Panico M, de Franchis R, Kraus JP, Andria G (1995) The molecular basis of homocystinuria due to cystathionine beta-synthase deficiency in Italian families, and report of four novel mutations. Am J Hum Genet 56: 1324-1333; Federico II U, Naples, Italy, 18 CBS-- cases, clinical findings, genetics. p 106

Kluijtmans LAJ, Boers GHJ, Stevens EWB, Ranier WO, Kraus JP, Trijbels FJM, van den Heuval LPWJ, Blom HJ (1996) Defective cystathionine beta-synthase regulation by S-adenosylmethionine in a partially pyridoxine responsive homocystinuria patient. J Clin Invest 98(2, July): 285-289; U Hospital Nijmegen, Nijmegen, the Netherlands, 1 CBS-- -case, improvement with treatment, genetics. p 106

Cordoba-Porras A, Sanchez-Quesada JL, Gonzalez-Sastre F, Ordonez-Llanos J, Blanco-Vaca F (1996) Susceptibility of plasma low- and high-density lipoproteins to oxidation in patients with severe hyperhomocysteinemia. J Mol Med 74: 771-776; U Antioquia, Medellin, Columbia, 6 CBS--, mean age 13y, 6 Controls mean age 13y, LDLTBARS, HDLTBARS, LDL%eNeg, LDLOx, HDLOx, Met, tCys. p 107

Mandel H, Brenner B, Berant M, Rosenberg M, Lanir N, Jakobs C, Fowler B, Seligston U (1996)a Coexistence of hereditary homocystinuria and Factor V Leiden – effect on thrombosis. NEJM 334: 763-768; Rambam Medical Center, Haifa, Israel, 6 CBS-- cases, 4 MTHFR-- cases, 1 cbl(C/D) case, thrombosis, Factor V Leiden. p 108

Quere I, Lamarti H, Chadefaux-Vekemans B (1996) Letter to the editor about Mandel et al (1996)a, also presenting their own case ; NEJM 335(4): 289; St Eloi Hospital & Necker Hospital, France; 15 CBS—cases, FVLeiden, thrombosis. p 108

Mandel H, Brenner B, Berant M et al (1996)b Letter to the editor responding to letter about Mandel et al (1996)a. NEJM 335(4): 290; Rambam Medical Center, Haifa, Israel; CBS--, FVLeiden, thrombosis. p 109

Dawson PA, Cochran DA, Emmerson BT, Kraus JP, Dudman NP, Gordon RB (1996) Variable hyperhomocysteinemia phenotype in heterozygotes for the Gly307Ser mutation in cystathionine beta-synthase. Aust NZ J Med 26(2 (April)): 180-185; U Queensland, Brisbane, Queensland, Australia, 1 CBS-- case and family members screened for gene, free versus total cysteine. p 109

van der Molen EF, Hiipakka MJ, van Lith-Zanders H, Boers GHJ, van den Heuvel LPWJ, Mannens LAH, Blom HJ (1997) Homocysteine metabolism in Endothelial cells of a patient homozygous for cystathionine beta-synthase (CS) deficiency. Thromb Haemost 78: 827-833; U Hospital St Radboud, Nijmegen, Netherlands, 1 CBS—case, endothelial cell line, &, >=3 normal cell lines, folinic acid, pyridoxine, homocysteine, coagulation. p 110

Watanabe T, Ito M, Naito E, Yokota I, Matsuda J, Kuroda Y (1997) Two siblings with vitamin B6-nonresponsive cystathionine beta-synthase deficiency and differing blood methionine levels during the neonatal period. J Med Invest 44: 95-97; U Tokushima School of medicine, Tokushima, Japan, 2 CBS-- siblings, betaine. p 111

Surtees R, Bowron A, Leonard J (1997) Cerebrospinal fluid and total homocysteine and related metabolites in children with cystathionine beta-synthase deficiency : the effect of treatment. Pediatr Res 42(5) : 577-582; Institute of Child Health, London, UK, 10/5 CBS-- cases versus controls, glycine, serine, 5-MTHF, methionine, SAM, CSF, betaine. p 112

Wilcken DEL, Wilcken B (1997) The natural history of vascular disease in homocystinuria and the effects of treatment. J Inher Metab Dis 20: 295-300; Prince Henry, Prince of Wales, and New Children's Hospitals, Sydney, NSW, Australia, 40 CBS--, 17 VitB6-responsive, 15 VitB6-nonrespon, Age 30, (9-66) years, 8 VitB6?, age?, treatment efficacy study, synthetic diet unacceptability to CBS-- patients not treated from birth. p 113

Bonham JR, Moat SJ, Allen JC, Powers HJ, Tanner MS, McDowell, Bellamy MF (1997) Free homocysteine may be a poor measure of control. J Inher Metab Dis 20 (Suppl 1): 20; U Wales College of Medicine, Cardiff, UK, 12 CBS-- cases, 13 CBS-+ obligate heterozygotes, 22 normal controls, total versus free homocysteine. p 114

Lobo CA, Millward SF (1997) Homocystinuria: a cause of hypercoagulability that may be unrecognized. J Vasc Interv Radiol 9(6): 971-975; Ottowa Civic Hospital, Ottowa, Canada, 1 CBS--?? case, thrombosis. p 115

Naughten ER, Yap S, Mayne PD (1998) Newborn screening for homocystinuria: Irish and world experience; Eur J Pediatr 157 (Suppl 2): S84-S87; global CBS-- prevalence reviewed. p 115

Yap S, Naughten E (1998) Homocystinuria due to cystathionine beta-synthase deficiency in Ireland: 25 years' experience of a newborn screened and treated population with reference to clinical outcome and biochemical control. J Inher Metab Dis 21: 738-747; The Children's Hospital, Dublin Ireland, All 25 CBS-- cases detected in Ireland 1971-1996. p 116

Walter JH, Wraith JE, White FJ, Bridge C, Till J (1998) Stratagies for the treatment of cystathionine beta-synthase deficiency : the experience of the Willink Biochemical Genetics Unit over the past 30 years. Eur J Pediatr 157 (Suppl 2) : S71-S76; Royal Manchester Children's Hospital, Manchester, UK, All 31 CBS-- cases diagnosed/managed since 1962, poor dietary compliance in patients not treated from birth. p 119

Kluijtmans LAJ, Boers GHJ, Kraus JP, van den Heuvel LPWJ, Cruysberg JRM, Trijbels FJM (1998) The molecular basis of cystathionine beta-synthase deficiency in Dutch patients with homocystinuria: effect of CBS genotype on biochemical and clinical phenotype and on response to treatment. Am J Hum Genet 65: 59-67; U Hospital Nijmegen, Nijmegen, The Netherlands, 24 CBS--Dutch cases, 23/24 VitB6-responsive, possibly not all of Dutch CBS--cases known at the time. p 123

Megnien J-L, Gariepy J, Saudubray JM, Nuoffer JM, Denarie N, Levensen J, Simon A (1998) Evidence of carotid wall hypertrophy in homozygous homocystinuria. Circulation 98(21 (Nov 24)): 2276-2281; Broussais & Necker Hospitals, Paris, France, 14 (10 CBS-- cases, + 4 ?-- cases), 15 their controls, 15 CBS-+ obligate heterozyg parents, 15 their controls, age, smoking, HT, diabetes, hypercholesterolemia, IMT, Diam, CSA-IMC. p 124

De Stefano V, Dekou V, Nicaud V, Chasse JF, London J, Stansbie D, Humphries SE, Gudnason V (1998) Linkage disequilibrium at the cystathionine beta synthase (CBS) locus and the association between genetic variation at the CBS locus and plasma levels of homocysteine. The Ears II Group. European Atherosclerosis Research Study. Ann Hum Genet 62(6 (Nov)): 481-490; 11 European Countries, 785 individuals participating in the European Athero-sclerosis Research Study II (EARSII), from 11 countries across Europe. p 125

Kluitjmans LAJ, Boers GH, Kraus JP, van den Heuvel LP, Cruysberg JR, Trijbels FJ, Blom HJ (1999) The molecular basis of cystathionine beta-synthase deficiency in Dutch patients with homocystinuria: effect of CBS genetype on biochemical and clinical phenotype and on response to treatment. Am J Hum Genet 65(1 (July)): 59-67; U Hospital Nijmegen, Nijmegen, The Netherlands, 29(27) CBS-- cases, Dutch, very roughly an even mixture of VitB6-responsive and non-responsive, treatment outcomes. p 126

Peter-schmitt MJ, Simmons JR, Levy HL (1999) Reduction of false negative results in screening of newborns for homocystinuria. NEJM 341(21): 1572-1576; New England Newborn Screening Program, Boston, USA, USA newborn screening. p 128

Yap S, O'Donnel KA, O'Neill C, Mayne PD, Thornton P, Naughten E (1999) Factor V Leiden (Arg506Gln), a confounding genetic risk factor but not mandatory for the occurrence of venous thromboembolism in homozygotes and obligate heterozygotes for cystathionine beta-synthase deficiency. Thromb Haemost 81: 502-505; The Children's Hospital, Dublin, Ireland (assume from their 1998 work see above), all 26 CBS--detected in Ireland 1971-97/98?. p 128

Yap S, Naughten ER, Wilcken B, Wilcken DEL, Boers GHJ (2000) Vascular complications of severe hyperhomocysteinemia in patients with homocystinuria due to cystathionine beta-synthase deficiency: effects of homocysteine-lowering therapy.Sem Thromb Hemost 26(3): 335-340; Meta-analysis Irish, Australian & Dutch, 84 CBS-- cases. p 129

Tangerman A, Wilcken B, Levy HL, Boers GH, Mudd SH (2000) Methionine transamination in patients with homocystinuria due to cystathinine beta-synthase deficiency. Metabolism 49(8): 1071-1077; various sources of patients, possibly The Netherlands, Australia, USA, & elsewhere; 22 CBS-- cases, selected to have higher methionine levels. p 129

Lentz SR, Erger SR, Dayal S, Maeda N, Malinow MR, Heistad DD, Faraci FM (2000) Folate dependence of hyperhomocysteinemia and vascular dysfunction in cystathionine beta-synthase-deficient mice. Am J Physiol Heart Circ Physiol 279: H970-H975; U Iowa, Iowa, USA, CBS++ & CBS-+ littermate mice, folate, homocysteine, aortic acetylcholine response. p 130

Weiss N, Heydrick S, Zhang YY, Bierl C, Cap A, Loscalzo J (2001) Cellular redox state and endothelial dysfunction in mildly hyperhomocysteinemic cystathionine beta-synthase-deficient mice. Arterioscler Thromb Vasc Biol 2: 34-41; Boston U School of Medicine, Boston, USA, CBS++ & CBS-+ littermate mice; vasodilation, cysteine, glutathione oxidation. p 130

Davi G, Di Minno G, Coppola A, Andria G, Cerbone AM, Madonna P, Tufano A, Falco A, Marchesani P, Ciabattoni G et al (2001) Oxidative stress and platelet activation in homozygous homocystinuria. Circulation104 : 1124-1128; U Chieti, U Naples Federico II, Italy, 13 CBS-- cases, 12 VitB6-responsive, 1 nonresponsive, lipid peroxidation, VitE, VitB6, betaine, treatment outcome. p 131

Trondle U, Sunder-Plassmann G, Burgmann H, Buchmayer H, Kramer L, Bieglmayer C, Hori WH, Fodinger M (2001) Molecular and clinical characterisation of homocystinuria in two Austrian families with cystathionine beta-synthase deficiency. Acta Med Austriaca 28: 145-151 ; U Vienna, Vienna, Austria, 2 CBS-- cases VitB6-semi/non-responsive, genetics, VitB6, folate, cobalamin, betaine . p 131

Yap S, Boers GHJ, Wilcken B, Wilcken DEL, Brenton DP, Lee PJ, Walter JH, Howard PM, Naughten ER (2001a) Vascular outcomes in patients with homocystinuria due to cystathionine beta-synthase deficiency treated chronically A multicenter observational study. Arterioscl Thromb Vasc Biol 21: 2080-2085; Meta-analysis Irish, Australian Dutch and UK; 158 CBS--cases; multicentre treatment outcome (thrombosis) study. p 132

Yap S, Rushie H, Howard PM, Naughten ER (2001b) The intellectual abilities of early-treated individuals with pyridoxine-nonresponsive homocystinuria due to cystathionine beta-synthase deficiency. J Inher Metab Dis 24: 437-447; The Children's Hospital, Dublin, Ireland, 23 CBS-- VitB6-nonresponsives, (13 early-detected good-compliance, 6 early-detected poor-compliance, 2 detected at 2years good-compliance, 2 untreated at 22, 12years, & 10 sibling controls, IQ,VitB6, VitB12, folate, betaine. p 133

Kalkanoglu HS, Coskun T, Aydogdu SD, Togatli A, Gurgey A (2001) Factor V Leiden mutation in Turkish patients with homozygous cystathionine beta-synthase deficiency. J Inherit Metab Dis 24: 367-369; Hacettepe U, Ankara, Turkey, 6 CBS--cases, FVLeiden, thrombosis. p 133

Sokolova J, Janosikova B, Terwilliger JD, Freiberger T, Kraus JP, Kozich V (2001) Cystathionine beta-synthase deficiency in central Europe: discrepancy between biochemical and molecular genetic screening for homocystinuric alleles. Hum Mutat 18(6 (Dec)): 548-549; Charles U, Prague, Czech Republic, population prevalences of CBS--, genetics, newborn screening underdetection. p 133

Gaustadnes M, Wilcken B, Oliveriusova J, McGill J, Fletcher J, Kraus, Wilcken DE (2002) The molecular basis of cystathionine beta-synthase deficiency in Australian patients: genotype-phenotype correlations and response to treatment. Hum Mutat 20: 117-126; Prince of Wales Hospital, Randwick, The Children's Hospital, Sydney, Royal Brisbane Hospital, Brisbane, Women's and Children's Hospital, Adelaide, all Australia, 36 CBS-- cases, 13 VitB6-responsive, 2 VitB6-partially-responsive, 21 VitB6-nonresponsive; genetics, diagnostic pathologies. p 134

Levy HL, Vargas JE, Waisbren SE, Kurczynski TW, Roeder ER, Schwartz RS, Rosengren S, Prasad C, Greenberg CR et al (2002) Reproductive fitness in maternal homocystinuria due to cystathionine beta-synthase deficiency. J Inherit Metab Dis 25: 299-314; Various USA & Canada, 11 CBS-- mothers, & offspring; low-methionine diet, VitB6, folate, VitB12, betaine. p 135

Pullin CH, Bonham JR, McDowell LFW, Lee PJ, Powers HJ, Wilson JF, Lewis MJ, Moat SJ (2002) Vitamin C therapy ameliorates vascualr endothelial dysfunction in treated patients with homocystinuria. J Inherit Metab Dis 25:107-118; Various, UK, 5 CBS-- cases & controls; VitC, endothelium-dependent flow-mediated vasodilation, BP, HR, cysteine. p 136

Atalay S, Akar N, Tutar HE, Yilmaz E (2002) Factor V 1691 G-A mutation in children with intracardiac thrombosis: a prospective study. Acta Paediatr 91: 168-171; Ankara U, Turkey 13 consecutive intracardiac thromboses, FVLeiden heterozygosity, indwelling catheter, ventriculoatrial shunt for hydrocephalus, cardiomyopathy, CBS--, tetralogy of Fallot, sepsis. p 138

Kruger WD, Wang L, Jhee KH, Singh RH, Elsas LJ (2003) Cystathionine beta-synthase deficiency in Georgia (USA): correlation of clinical and biochemical phenotype with genotype. Hum Mutat 23: 434-441; Fox Chase Cancer Center, Philadelph., Pennsylvan. USA, 12 CBS-- cases from 11 different families, from Georgia, USA, including 4 African-American, genetics, pathology. p 138

Kelly PJ, Furie KL, Kistler JP, Barron M, Picard EH, Mandell R, Shih VE (2003) Stroke in young patients with hyperhomocyteinemia due to cystathionine beta-synthase deficiency. Neurology 60: 275-279; Massachusetts General Hospital, Boston, Massachusetts, USA, 3CBS-- cases without usual stigmata, genetics, thrombosus,, arterial dissection, stroke. p 139

Yap S (2003) Classical homocystinuria: vascular risk and its prevention. J Inherit Metab Dis 26: 259-265. Children's University Hospital, Dublin, Ireland, 158 CBS-- Irish, Australian Dutch and UK, much as per Yap et al (2001a), see above. p 140

Orendac M, Zeman J, Stabler SP, Allen RH, Kraus JP, Bodamer G, Stockler-ipsiroglu S, Kvasnicka J, Kozich V (2003) Homocystinuria due to cystathionine beta-synthase deficiency : novel biochemical findings and treatment efficacy. J Inherit Metab Dis 26 : 761-773; Charles U, Prague, Czech Republic, 9 or 5 CBS-- cases, genetics, tHcy, SAH, Met, SAM, SAM/SAH, cystathionine, tCys, guanidinoacetate, creatine, N-methylglycine, N,N-dimethylglycine, serine, glycine, glutathione. p 141

Linnebank M, Junker R, Nabavi DG, Linnebank A, Koch HG (2003) Isolated thrombosis due to the cystathionine beta-synthase mutation c.833T>C (1278T). J Inherit Metab Dis 26(5): 509-511; U Munster, U Bonn, Germany, stroke and sinus thrombosis patients, apparently unselected ? p 143

Sueyoshi E, Sakamoto I, Ashizawa K, Hayashi K (2004) Pulmonary and lower extremity vascular lesions in a patient with homocystinuria: radiologic findings. AJR 182 (March): 830-831; Nagasaki U, Nagasaki, Japan, 1 CBS-- case; thromboses. p 143

Singh RH, Kruger WD, Wang L, Pasquali M, Elsas LJ (2004) Cystathionine beta-synthase deficiency: effects of betaine supplementation after methionine restriction in B6-nonresponsive homocistinuria. Genet Med 62 (March/April): 90-95; Emory U, Atlanta, Georgia, USA, 5 CBS-- cases, low-methionine high-cysteine diet, VitB6, betaine, cysteine. p 144

Devlin AM, Hapour L, Gholkar A, Fernandes, Ramesh V, Morris AAM (2004) Cerebral edema associated with betaine treatment in classical homocystinuria. J Pediatr 144: 545-548; Newcastle General Hospital, Newcastle-upon-Tyne & Willink Bio-chemical Genetics Unit, Royal Manchester Children's Hospital, Manchester,Both UK, 1 CBS-- case; betaine, cerebral edema. p 145

Vilaseca MA, Cuartero ML, Martinez de Salinas M, Lambruschini N, Pinto X, Urreizti R, Balcells S, Grinberg D (2004) Case report Two successful pregnancies in pyridoxine-nonresponsive homocystinuria. J Inherit Metab Dis 27: 775-777; U Hospital St Joan de Deu, Barcelona, Spain, 2 CBS-- siblings, VitB6-non-responsive, pregnancy experience of one; genetics, folate, betaine, cysteine. p 146

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Tables for Chapter 4

Homocystinuria due to Cystathionine Beta Synthase deficiency: Literature numerical and word-descriptive data extraction

Homocysteine is found in the urine as the homocysteine-homocysteine dimer called homocystine, and as the homocysteine-cysteine mixed disulphide, along with a significantly smaller amount of free homocysteine. In blood (i.e. plasma), there is also homocysteine bound to proteins.
Algorithms for calculating approximate total plasma homocysteine (tHcy) include:
Bonham et al (1997): tHcy = 60uM + 4.5 * (Hcy(ine) <20uM) + 2*(Hcy(ine)>20uM), and
Wiley et al (1989) tHcy = 40uM + fHcy rule (where fHcy means 'free' (not bound to protein, including homocysteine-cysteine mixed disulphide, homocystine (homocysteine-homocysteine dimer), and homocysteine by itself). Note the Bonham algorithm does not include Hcy-Cys, factoring (4.5, 2) for it.

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease		Hcy level	Treatment and biochemical etc contexts
McDonald L et al (1964); Lancet; Various Hospitals UK		PlateletCount(per ul)	PlateletStickiness(%remaining@20min	Hcy-uria present, Equates to tHcy > 40uM	Not provided?
	5CBS-- aged 4-15y (cites Carson et al 1963)				
	PB July 11	338,000	45%		
	PB July 12	327,000	48%		
	B	241,000	58%		
	C	380,000	60%		
	D	311,000	48%		
	E	253,000	48%		
	Av(B,C,D,E)	296,000	54%		
	5 Controls healthy, "of a similar age"				
	? July 11	306,000	51%		
	? July 12	327,000	50%		
	F	261,000	85%		
	G	137,000	77%		
	H	280,000	85%		
	I	185,000	75%		
	Av(F,G,H,I)	216,000	80%		
		Note: The possibility that correction for platelet count might decrease the observed difference in platelet stickiness to little or nothing requires consideration, which the authors do not address….(they cite the Platelet Stickiness method of Wright 1941, McDonald and Edgill 1957, 1958)			

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatment and biochemical etc contexts
Gibson JB et al (1964); J Clin Path; Two hospitals, Belfast, Ireland	7 males and 3 females from 7 families, age 1.5 – 32years at detection Case 1	All mentally retarded, IQ range up to 53 at diagnosis, 9/10 fair-haired and –complexioned, high malar flush and ectopia lentis (displaced eye lenses) regularly present, high-stepping spastic gait, high longitudinal foot arches and knock-knees. 3/10 kyphoscoliosis and chest deformity with arachnodactyly ("spider-fingers") (Marfanoid Habitus), of which: JR, male, considered normal at birth till 13months, when, vomiting, convulsions, coma, permanent rhs hemiparesis, diffuse cerebral atrophy demonstrated by air encephalography, dural thrombophlebitis diagnosed, only 1 carpal centre of ossification vs normal 2. At age 5years mental age 2years, unaided walking difficult, fingers short and stubby, sensitive to sunlight. Thereafter, a few epileptiform attacks, died age 7years following a few grand mal and Jacksonian seizures, followed by pyrexia. Necropsy: Skull asymmetrical slight rhs posterior bulge, pulmonary artery shows slight transverse intimal ridging, some small shock hemorrhages in left ventricular endocardium, endocardium of left atrium and mitral valve cusps slightly thickened by fibrous tissue, coronary arteries normal, liver mottled and congested (fatty), spleen firmer than usual under puckered regions of capsule, left kidney swollen, hemorrhagic, almost completely infracted, renal artery totally blocked by mixed thrombus, aorta slightly dilated in ascending part, aortic arch moderately dilated, Aortic wall stretched and thin as far as the left subclavian artery where lumen narrows and wall is thickened and encircled by 2 low band-like elevations of the lining each approx 2mm broad lying above and below the mouth of the subclavian "Just below it are a few wispy strands and tiny depressions of the intima; the vessel is unduly friable around them", aorta again slightly dilated down to the level of the renal arteries, where narrow line of transverse striations like comb marks extend for a few centimetres, the lower part of which covered by laminated mixed thrombus several mm thick till about the level of the cceliac axis, another such area (0.5 * 1.5cm) of transverse striation on anterior aorta just above mouth of inferior mesenteric artery, lowest part of aorta occluded by adherent thrombus which is chiefly red and extends into the common iliac arteries, mixed thrombus occlusion of coeliac axis into proximal hepatic artery, coeliac artery wall thick, splenic artery wall very thick and lumen minute, superior mesenteric artery at aortic mouth dilated and thickened following which dilation to thrice normal diameter in a fusiform aneurysm about 2cm long, mixed thrombus in lhs renal artery from kidney to aorta where vessel walls thick and fibrous, rhs renal artery thick walled, common iliac arteries have thick fibrous walls, dura around foramen magnum and upper spinal cord thicker and more fibrous than normal, superior sagittal sinus extensively obstructed by recanalized fibrous tissue, left cerebral hemisphere smaller than right particularly in occipital lobe, subdurally cortex brown-tinged and a little firmer than normal, left temporal lobe and inferior parietal lobe atrophied posteriorly so that the cortical pattern is small, occipital lobe many areas of brownish healed infarction disposed in lower layers of the cortex in roughly laminar distribution, orange-brown cystic softening (1.5 * 1.0cm in posterior left thalamus, a few minute cysts in left putamen, leptomeninges a little thickened in cervical region. (continues)	Hcy-uria present, Equates to tHcy > 40uM	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
(continued) Gibson JB et al (1964); J Clin Path; Two hospitals, Belfast, Ireland	Case 1 (cont)	(continued) (Histology) "The fibrous tissue of the aortic valve ring shows diffuse metachromatic staining. The pulmonary valve ring is similarly and less markedly affected" (thionine in McIlvane's buffers, metachromasia corresponds with that in alcian in paraffin sections), early bronchopneumonia in lungs, interstitial fibrosis apparently the result of old partial infarction, in spleen, liver cells generally atrophied, some with watery vacuolation, in the pale areas noted grossly fatty infiltration of all except the periportal areas, the old softenings of the brain are healed and surrounded by a moderate degree of fibrillary gliosis, no specific lesions from other areas of brain and spinal cord, slight gliosis and swelling of oligodendroglia recognizable throughout, a few small nodules of bone in fibrosed areas of the dura, "The chief lesions are vascular. The superior sagittal sinus and some of its immediate tributaries are occupied by fully organised and partly recanilized tissue. The venae cavae are normal. In the left renal vein there is moderate degree of fibrous endophlebitis. Other veins are normal. Medium-sized muscular arteries are affected in a patchy fashion. Lesions are detected in some of the arteries in the spleen, pancreas, colon, thyroid, and thymus, pelvis and left kidney, with minor or few changes of the same type in arteries of the heart, liver and right kidney. The internal elastic lamina is prominent and often thick. The main element is intimal fibrosis without deposits of iron pigments or other special features. The fibrosis is either symmetrically distributed so as sometimes to cause severe narrowing, as in the splenic artery, or symmetrically distributed in the form of pads. In the intimal pads, layers of elastic fibrils have split of from the main elastic lamina and small degrees of metachromasia are present. The medial coats of these arteries do not usually stain metachromatically but in some sections of the left renal and splenic arteries the muscle fibers are separated from each other and thinned out by prominent deposits of interstitial material which stains as collagen and also stains metachromatically with moderate intensity. The external elastic lamina is often swollen. The adventitia is little affected except in vessels such as the proximal part of the hepatic artery where thrombus is being organized; in those sites the vasa vasorum are congested and there is little polymorphic infiltration of the media…..There is slight concentric intimal fibrosis which is marked in the common iliacs and associated there with some fatty atheromatous plaques. Some area of intimal fibrosis stain well with alcain. The media is a little less cellular than normal with increased amounts of interstitial material between elastic fibres….The elastica is frayed, attenuated or irregularly thickened and disorderly in arrangement in such areas, particularly in the superior mesenteric artery. A little polymorph infiltration is associated with the aneurysmal dilatation ofthis vessel….The microscopic structure of the aorta is most altered in the arch above the left subclavian artery where the wall is stretched and thin. The intima of the arch is little altered. The adventitia is congested and the vasa vasorum are prominent; there is a little lymphocytic infiltration and no fibrosis. The media is deeply penetrated by by vasa vasorum and slightly fibrosed and the lines of the muscular and elastic fibres are disturbed and disrupted here and there. In little cysts or pools around some of the vasa vasorum and in little clumps between cells lie small granular or hyaline areas which are brightly metachromatic…….mouths of the renal arteries and neighboring large branches. Here the findings are complicated by ridges of intimal fibrosis. These ridges and striations are composed of moderately cellular fibrous tissue thickly interspersed with elastic fibrils. Staining for fibrin and for iron pigment is negative in the ridges and metachromatic staining is generally slight except where the fibrous tissue merges with the the process of organisation of thrombus in the aortic lumen. The media is not extensively altered but is thinned under the intimal ridges and there the vasa vasorum are prominent and penetrate deeply into the media. The adventitia is slightly fibrosed and there is a little lymphocytic infiltration round the vasa. The pulmonary artery is of looser texture than normal and between the fibres lie many small deposits of material that stain metachromatically." (continues)	Hcy-uria present, Equates to tHcy > 40uM	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
(continued) Gibson JB et al (1964); J Clin Path; Two hospitals, Belfast, Ireland	(Cont) Case 2	(Continued) GM, detected at the age of 13years, mentally retarded, Marfanoid Habitus, ectopia lentis, hematuria with oedema of the upper eyelids at age 6years "; blood pressure was 115/70mmHg, blood urea 168mm%. The subsequent course of his renal disease and the persistent hypertension that he developed have been described elsewhere (Loughridge, 1959, case 4). ..aged 13years, he was readmitted to hospital; his blood pressure was 170/120mmHg at the lowest reading. The left kidney, which was ectopic, was removed surgically.", kidney97g, pale, capsule outer surface slightly granular, texture a little firmer than normal, architectural pattern of cut sections slightly blurred, no infarcts, main renal artery and some of the interlobular arteries small in calibre and walls symmetrically thickened, venous lesions absent, no stones. (Histology) glomerular lesions not seen, previous glomerulonephritus not indicated, "Fibrinoid change has not occurred in glomerular afferent arterioles; a few are slightly fibrosed…..The arteries in general have undergone a little symmetrical medial hyperplasia, but irregular asymmetrical proliferation of fibrous and other elements is commoner and more marked, particularly in the vessels of medium calibre. In the larger arteries, fibrous tissue has proliferated in the intima as a thin irregularly disposed layer and elastic fibres extent into it from the internal elastic lamina; the intima shows marked metachromasia. In the media muscle fibres are often enlarged and somewhat irregularly arranged with an increase of interstitial material which stains as collegen and is, in places, metachromatic; fine elastic fibrils of frayed appearance are scattered through the muscle singly or occasionally together in narrow wedge-shaped segments of the vessels. The external elastic lamina is a little thickened and frayed; there is little or no alteration in the adventitia; sometimes a little metachromasia is seen. Similar changes have taken place in the arcuate arteries. At this level, intimal fibrosis is often marked in the form of pads and other asymmetrical deposits. Many elastic fibrils traverse these deposits and the internal elastica is often reduplicated. Sometimes the muscle is irregularly disposed and segmental area of fibrosis occur in the media. The walls of the straight arteries are thickened by fibrosis of the media, sometimes severely, the internal elastica is often reduplicated, and there is often a little ring of metachromasia beneath it and hyperplasia of the muscle." For other details see GM case of Carson et al (1965) below	Hcy-uria present, Equates to tHcy > 40uM	
	Case 3	AS, male, severely mentally retarded, Marfanoid Habitus, ectopia lentis, several unexplained episodes of venous thrombosis in the lower limbs from the age of 2years 8months, at age 7years admitted to hospital with venous thrombosis in the lower limbs, liver enlarged and palpable 3or4 fingerbreadths below costal margin, platelets "248,000/c.mm.", prothrombin 30%, Wassreman and Kahn reactions negative, generalized aminoaciduria, generalized osteoporosis, renal tubular dysfunction resemblant Fanconi Syndrome, metabolic acidosis, hypopotassemia, steatorrhea, laparotomically, liver markedly enlarged, soft yellowish-pink with a smooth surface and rounded edge, "In a surgical biopsy of liver, a marked degree of fatty change was seen. Fatty change extended from the centre of each lobule almost as far as the portal tracts and it was only round the portal tracts that the liver cells had their normal form. Some of the fatty cells were grossly enlarged." Died of unknown cause aged 8years – necropsy not obtained.	Hcy-uria present, Equates to tHcy > 40uM	Cystine not present
	Case 4	PB, female, age 6.5years, mentally retarded, ectopia lentis, liver enlarged with biopsy as per Case 3.	Hcy-uria present, Equates to tHcy > 40uM	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Gerritsen et al (1964) ; U Wisconsin, USA		Provides the biochemical details for the case RS of Chou SM et al (1965) ; Arch Path; U Wisconsin, USA, See therein Immediately below.		
Chou SM et al (1965) ; Arch Path; U Wisconsin, USA And case detail from Gerritsen et al (1964) ; U Wisconsin, USA	1 Case: CBS-- or cbl— ?????..	RS, male, failure to thrive, poor developmental milestones, knock-knees, at age 2months extreme spasticity/rigidity and marked hyper-reflexia, Rossolimo, Babinski, grasping, rooting, sucking, gag, Moro reflexes ok, Abdominal, Magnus and De Kleijn neck reflexes absent, No reaction to light and little to sound, (Gerritsen et al 1964( )) Homocystinuria in conjunction with higher plasma methionine (21,23mg/dl vs 0.5mg/dl), lower urinary cysteine (trace*4 vs 1.8mg/day), and lower urinary cystathionine (<1mg/day*4 vs 0.8mg/day) than a normal control, at baseline, despite varying non/ corroborative from various biochemical loading tests, strongly confirms RS to be a CBS—case rather than one of the other homocystinurias. Terminally, signs of severe systemic infections, death at age 1y being thought due to toxic sepsis, severe diarrhea, and respiratory difficulties. Autopsy: Body markedly underdeveloped, undernourished, dehydrated, Genitalia markedly hypoplastic, testes in inguinal canals, lungs with multiple foci of hemorrhage and abscess formation, thromboembolism within the right major pulmonary artery, iliac mucosa covered with acute inflammatory exudateand with focal hemorrhages and hyperemia, liver parenchyma contained mottled yellow areas probable representing fat, hepatocytes with numerous fine vacuoles, adrenal medulla congested and hemorrhagic in some areas, no alterations observed in vascular walls in sections studied with Van Gieson stain., brain weight 620g (66% normal/age), micropolygyria throughout vertex (absent from undersurface), very thin olfactory bulbs, normal cerebellum and brain stem, Sylvian fissures closed and operculum invisible, corpus collosum with marked hypoplasia, architecture of basal ganglia well maintained but obvious wasting of white matter, many microgyri present in cerebral cortex, subcortical white matter diffusely vacuolated with wide-spread absence of myelin, vacuoles empty, size 20-100u frequently lined by thin axons, a few macrophages containing sudanophylic substances seen along the small vessels, vacuolization most extensive at the junction of the gray and white matter appearing to extend diffusely into the white matter and to a lesser extent into the deep layer of the cortex, this change also observed in micopolygyric areas, corpus callosum relatively spared, loss of myelin always accompanied by vacuolation, in basal ganglia vacuolization present and extended thereto from the internal capsule and internal medullary lamina of the thalamus, milder such changes also observed in the subcortical white matter of cerebellum, (cerebellar?..) “Sulfhydryl and disulfide stains were used on sections from this patient as well as on similar sections from a 2y old child dead of other causes. In this control patient, the sulfhydryl and disulfide reactions were stronger in the white than in the grey matter. In RS, the white matter showed only very feint sulfhydryl reaction even in the nonvacuolated and myelinated areas; the disulfide reaction was absent. The reaction in the grey matter was relatively stronger than in the white, but was less than in the control, less marked vacuolization seen in brain stem’s central tegmental, coticospinal, corticobulbar, and corticopontine tracts, in the cervical spinal cord the vacuolization appeared most conspicuously at the junction of the grey and white matters “in spite of the presence of the severe spongy changes seen in the pyramids of the brain stem, both the ventral and lateral corticospinal tracts of the cord were less vacuolated.”, “No vascular lesions were observed in any of the vessels in any of the sections studied.”, From their discussion: “Indirect support for the hypothesis that this is not a primary demyelinating disease is provided by the absence of astrogliosis, absent gitter cell reaction, and remarkably well-preserved nerve cell bodies and axons. It is commonly supposed that the myelin loss and sponginess in this type of degeneration is secondary to serous imbibition, which in turn, is due to a congenital defect in the blood-brain barrier or osmotic dysfunction. The marked deficiency of sulfhydryl and disulfide groups at the site of the greatest sponginess suggests that any defect in the blood-brain barrier is due to the lack of sulfur proteins. However, there is no evidence for a lack of sulfur proteins in any of the other patients with spongy degeneration…..”	Hcy-uria present, Equates to tHcy > 40nM	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatment and biochemical etc contexts
Laster et al (1965); J Clin Invest; National Institutes of Health, Bethesda, Maryland, USA Note: Most of the details for CT and MAG had to be retrieved from Mudd et al (1964); Science, and, Finkelstein et al (1964); Science Note: Milk methionine and cysteine details derived from data from Kirschmann JD, Dunne LJ (date!!!); Nutrition Almanac 2ndEd Note: The estimations of CT's and MAG's daily requirement of methionine + cysteine are derived from Shils et al (1999), and a standard Periodic Table	CBS—homozygotes: CT, age 9y, female, white, CBS activity nil; MAG, paternal cousin of CT, age 25y, female, white, CBS activity 31"mumole/mg protein" Controls: 5 normal volunteers, and, SM, the maternal grandmother of CT, age 56y, white CBS activity 257 "mumole/mg protein"	Mental retardation, ectopia lentis, knock-knees Asymptomatic, no mental retardation, minimal focal fatty change on histology of liver biopsy Note (continues far rhs column): The baseline diets used in the study provided very similar amounts of methionine + cysteine (they provided approx equal weights of each; MW(Methionine) =149g/mol, MW(Cysteine) = 121g/mol) to those estimated daily requirements derived by me (far rhs column) for CT and MAG, who were both excreting nearly the same or a little less molar quantities of SO_4^{2-} in the urine. The only control with this detail provided, JRMcC, received .07mmol of (Met+Cys)/ kgBW/day but was excreting nearly twice that molar quantity of SO_4^{2-} in the urine. Capsules of Met/Cys were added to the baseline diets to make up the noted amounts (0.6 or 0.8 mmol/kgBW/day)	Hcy-uria present, 40-50mg per day, Equates to tHcy > (probably >>/>>>) 40uM Hcy-uria present, Equates to tHcy > 40uM	"During infancy, CT received only cow's milk, e relatively poor source of cystine." Cow's milk: .025gMethionine, .009gCysteine, per gProtein "For the first 6 weeks of life MAG received human milk, a richer source of cystine." Human milk: .021gMethionine, .019gCysteine, per gProtein **Study Results:** On a methionine intake of 0.6mmol/kg/day: MAG excreted SO_4^{2-} 0.1mmol/kg/day1 0.2mmol/kg/day2 Controls excreted SO_4^{2-} 0.4 mmol/kg/day1 0.5mmol/kg/day2 On a methionine intake of 0.8mmol/kg/day: CT excreted SO_4^{2-} 0.05 mmol/kg/day1 0.15mmol/kg/day2 Controls excreted SO_4^{2-} 0.6 mmol/kg/day1 0.7mmol/kg/day2 Note: CT's estimated (normal, age 9y) daily requirement of Methionine + Cysteine is 23mg/kgBW/day, Approx (23mg/kgBW/day) / (135mg/mmol, equal weighting in averaging the MW of Met, Cys) = 0.17mmol/kgBW/day MAG's estimated (normal, age 25y) daily requirement of Methionine + Cysteine is 13mg/kgBW/day, Approx (23mg/kgBW/day) / (135mg/mmol, equal weighting in averaging the MW of Met, Cys) = 0.10mmol/kgBW/day (continued 2 columns to left)

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatment and biochemical etc contexts
Carson NAJ et al (1965); J Pediatrics; 2 Irish and 1 English hospitals, UK	10 cases of homocystinuria discovered in a survey of 2,920 mentally retarded individuals in Northern Ireland:			
	Case1	PB, female normal gestation and birth, walked alone at 15months, at age 4y bilateral ectopia lentis, EEG normal, at age 4.7y generalized major motor seizure, at age 6y awoke one morning with a profound weakness in left leg (seizure occurrence unexcludable) with gradual only partial return of function, at age 6y keratoconjunctivitis retinal detachment proceeding quickly to near blindness, at age 6.2y another similar major motor seizure, at age 7.7y very fine fair hair, intense malar flush, moderate Marfanoid continuum, moderate bilateral upper motor neuron paresis, shuffling gait, glaucoma, cystic retinal detachment with pigmentary changes, height and weight at 35%ile, EEG shows diffuse generalized dysrythmia,	Hcy-uria present, Equates to tHcy > 40nM	At age 5.5y cystine 1g*3/day At age 6.2y cystine discontinued, At age 6.5y cystine 1g*3/day At age 6.8y cystine discontinued
	Case 3	SR, male, normal birth, at age 6m first sat up, at age 1.3y first walked, at age 2y intermittent swelling of the legs with enlargement of the abdomen and of the superficial abdominal veins, at age 2.5y ditto, at age 3y began to speak, at age 6y could use short sentences, history of frequent respiratory infections, at age 8y, fair-haired, intense malar flush with slight cyanotic tinge, ectopia lentis, enlarged abdominal and right inguinal varicose veins, knock knees and high foot arches, broad-based and clumsy gait, IQ 34.	Hcy-uria present, Equates to tHcy > 40nM	
	Case 4	JR (brother of SR), male, normal birth, at age 6y fair hair, intense malar flush, IQ<30, knock-knees and high foot arches, For autopsy details see JR case of Gibson et al (1964) above.	Hcy-uria present, Equates to tHcy > 40nM	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
(continued) Carson NAJ et al (1965); J Pediatrics; 2 Irish and 1 English hospitals, UK	(cont) Case 2	PB (sister of PB above), female, premature birth with pre-eclamptic toxemia, birthweight 1023g, at age 10m sat alone, at age 1.7y first walked, at age 3.5y generalized motor seizure of 3-6h duration, at age 4.7y generalized motor seizure of 3-6h duration, at age 4.9y generalized motor seizure of 3-6h duration, at age 6y EEG within normal limits, at age 9y "not yet able to put two words together." , striking physical resemblance to sister PB, severe fatty change of liver by biopsy, at 9.5y prophylactic removal of left eye lense, 2weeks later during apparently benign convalescence therefrom sudden cyanosis and death. Autopsy: Immediate cause of death massive pulmonary emboli in main pulmonary arteries, calibre and valve markings suggested a probable femoral/iliac venous origin but no residual thrombus detectable there, heart somewhat enlarged 145g, left atrium endocardium thickened and rather white, "Throughout the descending aorta and its main branches were patchy lesions which consisted of small transverse striations 1-2mm long and approx 0.5mm apart…apparently unrelated to vessel junctional and stress areas, At the beginning of the descending aorta, in an area of such striations, there was a bandlike elevation which narrowed the lumen and was apparently an advanced degree of this lesion. In the superior sagittal sinus, straight sinus, both transverse sinuses, and great cerebral vein and its tributaries there was some recent thrombus but no evidence of significant engorgement of the superficial cerebral veins" brain slightly small for age 1095g, slight malformation of right occipital lobe cerebral gyri, liver enlarged 1284g with mottled yellow appearance and fat droplets on section, detail for PB, JW (case Komrower et al 1963) vs Controls 1,2: Fat %: Myristic Palmitic Palmitoleic Stearic Oleic Linoleic Arachidonic Cases: 1.2, 0.8; 22,20; 6,2; 6,9; 60,58; 5,9; 0,1 Controls: 0.9,0.3; 29,34; 5,5; 14,13; 33,31; 14,12; 5,5 "Corresponding to the transverse intimal striations, areas of fibrous intimal thickening were seen in the thoracic and abdominal aorta, the common and external iliac arteries, coronary artery branches, and small-sized pulmonary arteries. In the aorta subjacent to the intimal striations there were areas of disruption and fragmentation of the elastic tissue. In the thoracic aorta in the area of narrowing of the lumen there was a large pyramid of hyaline tissue.", recent and organizing thrombus in dural sinuses, moderate endocardial fibrosis of left atrium, lung parenchyma showed some amphysematous changes, severe fatty liver change most marked centrilobularly (no cirrhosis), "Brain: Diffuse changes were mostly limited to minimal cell loss without reactive changes in the outer layers of the cerebral cortex. There was focal neuronal loss in the Ammon's horn and the end-folium of the hippocampus with some fibrous gliosis. Changes in the white matter were limited to some beading and unevenness of myelin sheaths. Areas in the midbrain and around the third and fourth ventricles showed a diffuse microglial proliferation. The most conspicuous lesions were focal areas of incomplete necrosis with disappearance of nerve cells and proliferation of microglia found in the basal ganglia, especially the thalamus. No thrombi or thrombotic remnants were seen in blood vessels within the brain substance but some of the areas of focal necrosis and gliosis were situated around small blood vessels and a fresh thrombus was present in a small branch of the posterior central artery.", \ "Eyes: The most striking pathologic change in the eyes was in the zonular fibres which were deficient in the region of the lens and appeared recoiled to the surface of the ciliary body where they lay retracted into a network which fused with the greatly thickened (~3*) basement membrane of the nonpigmented ciliary epithelium…Electron microscopic examination revealed zonular fibers which were disoriented and granular rather than fibrillar. There was peripheral cystic degeneration of the retinas", striking incidence of schizophrenia on the maternal side.	Hcy-uria present, Equates to tHcy > 40nM	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

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(continued) Carson NAJ et al (1965); J Pediatrics; 2 Irish and 1 English hospitals, UK	(cont) Case 5	GMcC, male, normal gestation birth neonatal, at age 8.5m first sat up unaided, at age 2.2y first stood unaided, never been able to bend over to pick up objects from floor nor to ascend or descend stairs, at age 5y 3%ile height & weight, fair hair, intense malar flush cyanotic on crying or exposure to cold, bilateral ectopia lentis, moderate Marfanoid continuum, speech limited to unintelligible sounds, intelligence assessed at <2y in all areas, right indirect inguinal hernia (repaired), bone age approx 2y "the long bones were thin with multiple Harris lines and wide irregular metaphyses.", electrocardiographically and roentgenologically supported right ventricular cardiomegaly, "an angiocardiogram revealed a difference in blood supply to the lower lobe of the left lung in that the peripheral arteries were less numerous and the veins smaller than on the opposite side.", "Two days following the catheterization there was a sudden onset of swelling of the right leg consistent with further venous obstruction, EEG sleep record normal, "slightly abnormal red fluorescence of the hair cuticle with acridine orange when examined under the ultraviolet microscope.", "Biopsy of the rectus abdominus muscle taken at the herniorrhaphy was normal."	Hcy-uria present, Equates to tHcy > 40nM	
	Case 6	AMcC (sister of GMcC), female, birth and neonatal normal, at age 6m first sat up unaided, at age 1.5y first walked unaided "although her gait is still a ducklike waddle.", not able to bend over to pick up objects from floor nor to ascend or descend stairs, "The past history was remarkable for the frequency of respiratory infection.", at 1.9y fine fair hair, intense malar flush, 10%ile height & weight, bilateral ectopia lentis, moderate Marfanoid continuum, "An EEG revealed a normal sleep pattern but "some of the bursts of fast activity were of high voltage and sharp form similar to those found in phenylketonuria."", electrocardiographically and roentgenologically supported right atrial cardiomegaly.	Hcy-uria present, Equates to tHcy > 40nM	
	Case 7	GM, male, at age 6y hematuria with eyelid edema, left kidney located in pelvis though no gross hydronephrotic changes, at age 8y attacks of nausea vomiting and weakness BP 150/100-175/120mmHg, fair hair, intense malar flush, livido reticularis on extensor surfaces of legs, bilateral ectopia lentis, moderate Marfanoid continuum, peculiar wide-based gait, IQ 50, "The corneal epithelium was slightly edematous and peripheral corneal vascularization was present. The anterior chamber angles were obliterated by peripheral anterior synechiae, while the iris was atrophic.Atrophy was evident in the ciliary body and the zonular fibres were thickened; a hyaline membrane covered the ciliary epithelium and the pars plane…Both the choroid and retina were atrophic, the latter showing absence of ganglion cells and cystic degeneration of the nerve fiber layer.", at 13.5y BP 200/140mmHg but no signs of congestive failure, "The renal artery and its branches showed changes similar to those found in arteries at the postmortem examination of cases 2 and 4." (For further autopsy details see GM case of Gibson et al (1964) above)	Hcy-uria present, Equates to tHcy > 40nM	

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(continued) Carson NAJ et al (1965); J Pediatrics; 2 Irish and 1 English hospitals, UK	(cont) Case 8	MMcG, female, at age 2y first walked, at age 6y first speech, at age 7y bilateral ectopia lentis, at age 29y IQ 25, looked much older than age, fine fair greying hair, malar flush with cyanotic tinge, livido reticularis on legs, Marfanoid habitus with arachnodactyly, unsteady shuffling gait, unable to ascend or descend stairs, at age 29y "died from what was thought to be a cerebral thrombosis	Hcy-uria present, Equates to tHcy > 40nM	
	Case 9	AS, male, birthweight 6lb slight neonatal jaundice, at age 3w cyanosis, at age 6w cyanosis, at age 2y not walking, at age 7.2y mentally retarded, intense malar flush with a cyanotic tinge, bilateral ectopia lentis, moderate Marfanoid continuum, dilated anterior abdominal varices, palpable liver "An open biopsy of the liver showed fatty change with centrilobar accentuation without fibrosis, prothrombin time 17seconds (30%), by age 8y "had numerous hospital admissions mainly for unexplained venous thrombosis of the lower limbs.", death.	Hcy-uria present, Equates to tHcy > 40nM	
	Case 10	RK, ("provisional" case initially described as Marfan Syndrome case) male, at age 8m first sat up unaided, at age 1.5y first walked unaided, at age 7y possible meningitis, at age 14y IQ 50, incapable of unaided walking, Marfanoid habitus with arachnodactyly, bilateral ectopia lentis, cataracts, blindness, at age 28y sudden cardiac failure and death	Hcy-uria present, Equates to tHcy > 40nM	
Brenton DP et al (1965a); Pediatrics; University College Hospital Medical School, London, UK	2 Hcy-uric child'n dying at age 9y from pulm'y embol after eye op, and, 14 Control dying of various other causes	The Case children are PB (see Carson et al 1965 above) and JW (of Komrower & Wilson 1963) "Inspection of Table III reveals the strikingly low concentrations of free cystathionine in all parts of the brains examined in homocystinurics (0.0-0.4 mg/100gWetW) as compared to normal brains from patients dying from entirely unrelated conditions" "If (n = 11) the adult control tissues alone are considered, the various areas of brain show striking differences in the concentration of free cystathionine from one area to another: Occipital lobe (12-90) > Frontal lobe (11, 13) > Pons and Medulla (8, 5) > lateral lobe of Cerebellum (3.5, 2)" "The other sulfur-containing free amino acids showed no difference either between controls and homocystinurics or between various areas" (Note: The ranges given for most of these values so very wide as to require explanation of themselves, and a ss conclusion is not available, which latter might nearly apply to the results for cystathionine as the results are very patchy and sparse) Note: although various unexpected results for ie enzyme activities raise questions as to the chemical/biochemical success of these works, in some support of the results is their citation of Gjessing et al (1963) reporting large concentrations of cystathionine in the urines of four patients with adrenal neuroblastoma, one subsiding with regression of the metastases.	Cases: Hcy-uria present, Equates to tHcy > 40nM	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Brenton DP et al (1995b); J Pediatrics; U College Hospital Medical School, London, UK	2 CBS--, aged 2-5y 1 CBS-+ Obligat het'zyg parent 3 Control normal adult males	Plasma Methionine and (free) Homocysteine Across Time Following Oral Methionine Loads of 0.1g/kgBW: Plasma: Met@: 0hours 6h 24h 72h 6d fHcy@: 0h 6h 24h 72h 130uM 1140 810 600 340 162uM 148 148 237 <70uM 740 <70 nil 22 nil <70uM 540 <70 Note: "<70" refers to a region on the the y axis not further differentiable by my eye. Note: The results of the analogous series of experiments using intravenous administration of the methionine, were very much in agreement with those for the oral administration noted above, with the obvious exception being the more immediate input into the bloodstream, and the more immediate commencement of the metabolism therefrom. Further Results: "Excretion of total homocystine (homocystine + homocysteine) and the mixed disulfide of cysteine and homocysteine (ug/mgN) before and after loads of methionine" Hcy(ine): 0-2hBefore 0-24hAfter Hcy-Cys: 0-2hBefore 0-24hAfter CBS--GMcC Age 5y: 6.8 14.0 5.9 10.7 CBS--AMcC Age 2y: 15.2 5.7 CBS--PB Age 7y: 10.5 21.6 6.7 14.0 Further Results: "Excretion of homocystine, homocysteine, and the mixed disulfide of cysteine and homocysteine, ug/mgN, before and after intravenous methionine administration to PB; values were derived from iodoacetate-treated urine specimens at the times shown" @: Basal 3-6hours 23-26hours 48hours 72hours 7days Hcy: 1.1 4.8 4.6 2.6 2.1 1.2 Hcy(ine) 8.8 8.8 20.6 12.0 8.5 13.8 CBS--PB Age 7y Hcy-Cys 6.7 19.3 11.6 5.8 3.6 4.4 Note: "by the automatic analyser method of Spackman, Stein and Moore (1958)" – difficult to explain the unexpectedly relatively high purportedly free Hcy?....Likewise the proportional reversal of Hcy(ine):Hcy-Cys at 3-6h?.....	fHcy (add >40uM for tHcy 6d 7d 237 274	unknown

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
Schimke RN et al (1965); JAMA; Various eastern USA, mostly examined at Johns Hopkins Hospital, Baltimore, Maryland, USA	38 CBS-- age 21y (3-45y) roughly normal distrib skewed a little to the young	"a program was initiated for screening urine from patients with ectopia lentis or presumed Marfan Syndrome, or both. In most cases urine was obtained by mail. The cyanide-nitroprusside test was used for screening purposes." The origin of the cases seems to have been the Eastern USA in general: Wilmer Ophthalmological Institute and from the files of the Division of Medical Genetics 143 families screened, 9 families ascertained); Columbia University Institute of Ophthalmology 30, 2; University of Pennsylvania 15, 3; University of Michigan 53, 1; various MDs 200, 0; Washington U 2 families ascertained; MD, Buffalo 1 family ascertained; Children's Hospital, Philadelphia 1 family ascertained, Indiana Medical School 1 family ascertained. "Patients with homocystinuria in each of the 20 families were examined, most of them at the Johns Hopkins Hospital, the others in the home or in an institution near their home." The following excerpts omit some detail that is generally in close agreement with information provided by other similar though sometimes more detailed studies dealt with by me elsewhere here: "The Skeleton - …..A feature unknown in the true Marfan Syndrome – generalised osteoporosis, usually leading to codfish vertebrae and some degree of vertebral collapse – is present in all cases…. The palate is frequently narrow and highly arched." "Cardiovascular System – Arterial or venous thrombosis and cutaneous flushing are the main cardiovascular features. Fatal coronary occlusion accurred in a 20year old woman who had angina for a year or more before death and electrocardiographic evidence of a previous posterior myocardial infarction. Well-documented anteroseptal myocardial infarction occurred at 31y of age in a woman alive aged 45. Death presumably from coronary occlusion occurred in an 18y old boy. Another 18y old boy and a 12y old girl died of bilateral thrombosis of the internal carotid arteries. Thrombosis in the terminal aorta, iliacs, and subclavian arteries was manifested by arterial bruits, loss of pulses, and ischemic symptoms. Renal artery narrowing with unilateral renal atrophy was demonstrated in an 18y old with hypertension, and similar involvement was suspected as causing hypertension in several other patients. An 8y old boy was essentially pulseless because of recurrent arterial thromboses. He had also suffered 2 cerebrovascular accidents and dilated abdominal collateral, suggesting thrombosis of the inferior vena cava, were apparent. Thrombosis of the inferior vena cava and pulmonary emboli were the causes of death in a 13y old girl. Recurrent thrombophlebitis in the extremities with pulmonary embolism occurred in some cases. A 28y old man died of portal vein thrombosis. Intracranial thromboses, venous and arterial, led to neurological signs, seizures, and in one case, the non-specific diagnosis of "cerebral palsy" from which death occurred at the age of 3… …Venipuncture and arterial puncture may initiate thrombosis. Arteriography and cardiac catheterization should probably be avoided and only essential surgery done. In the 2 patients dying of bilateral internal carotid thrombosis, acceleration of the thrombotic process by cerebral arteriograms is suspected." "Central Nervous System – A majority of the patients show mental retardation in some degree; however, 16 of the 38 patients were judged to have normal intelligence….. Many learn well by rote but have difficulties with exercises in reasoning……. Marked nervousness is a striking feature of most cases…… Electroencephalograms, obtained in 8 patients shoed abnormality in all, including 2 with normal intelligence. Seizures occurred in 3 patients, related probably to intracranial arterial or venous thromboses, or both." "Pathology – Large arteries such as the coronary, renal, subclavian, iliac, and carotid showed drastic medial changes with dilatation and thrombosis. The media was thin. The muscle fibers of the media were widely separated by expansion of the intercellular ground substance. Thrombosis and organisation without complete occlusion had occurred episodically, with formation of new internal elastic lamellae after each episode. The aorta showed an abnormal elastic weave in both longitudinal and transverse section. Thrombosis in the terminal aorta was present in one of the autopsied cases and was suspected clinically in another deceased patient. In at least 2 of the autopsies, extensive left atrial endocardial fibroelastosis was found. Fatty liver was present in all autopsied cases." There were 15/38 cases noted to have had thrombotic disease (13) or myocardial infarction (2), "presently or at death", and note the age distribution as noted above column to left.

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Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Harris ED Sjoerdsma A (1966) ; Lancet; National Heart Institute, Bethesda, Maryland USA	2 mental retard CBS--,	(age 22, 25y) 6mm skin punch biopsy from hip 3.9, 7.5% soluble collagen; 1.6, 1.5 alpha:beta	CBS--: Hcy-uria present, Equates to tHcy > 40nM	
	2 norm IQ CBS--,	(age 22, 25y) 6mm skin punch biopsy from hip 1.7, 2.5% soluble collagen; 1.2, 1.1 alpha:beta		
	34 control	(all adults: 5 no dis, 10 hypertension, 9 malign, 4 cardiac, 6 misc) " biopsy 1.4-3.5% soluble collagen; 0.8-1.2 alpha:beta	unknown	

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Authors, Journal, Site	Study Group	Disease	Hcy level	Treatment and biochemical etc contexts
Brenton DP et al (1966); Quart J Med; University College Hospital and Medical School, London, UK Note: The estimations of SM's daily requirement of methionine + cysteine (assuming normalcy of CBS, not the case) are derived from Shils et al (1999)	Case 1	SM, forceps delivery, early developmental milestones purportedly normal up to age 3years, from which time his mental performance "deteriorated and he lost the ability to count." (no history of unconsciousness, epilepsy, paralysis suggestive of thrombotic etiology), later private schooling, myopia at age 7y progressing rapidly next 3 years, ectopia lentis at age 10y two separate events into anterior chamber spontaneously replaced with bed rest, knock-knees, Marfanoid Habitus but no arachnodactyly, "Bilateral tibial osteotomies were done and he was immobilized in plaster of Paris. Over the next 3 months he developed leg oedema and engorgement of superficial veins of the chest, abdomen and thighs… .angiogram… thrombotic obstruction… heparin…subsided", at age 14y IQ (WIS) – Vocabulary and Reasoning 60-70 Performance Scale 44, "No abnormality was found in the electrocardiogram or electroencephalogram.", "Grossly increased" platelet stickiness (continued from far rhs column) SM's urinary methionine sulfoxide apparently (graph) went from approx 0 (if measured…?) to 300mg/day at the end of the period of substitution of methionine for cysteineand thereafter back down to 100mg/day and then to apparently (graph) 0 (if measured…?) during the period of substitution of cystine for 70% of the supplementary methionine. SM's Hcy: "free" (I think) fHcy = 90uM, =>140uM tHcy did not vary much throughout study: The magnitude suggests some VitB6-responsivity?…. as does case history. "The platelets appeared to be equally abnormal in the first, third and fourth periods and no decreased stickiness had occurred when the last measurement was made 2days before SM's discharge although the plasma methionine levels had been near normal values for 5days. The only change in platelet behaviour was in the second period (supplementary cysteine replaced with methionine) when temporarily they were more sticky than usual and clumped together to such an extent that it was not possible to count them" ?: Heparin sulfation issue?…..	Hcy-uria present, Equates to tHcy > 40uM	**Nitrogen Balance Study:** "The basic diet supplied 475mg Methionine and only about 85mg Cysteine per day…SM's diet was supplemented with 1g each of methionine and cystine in the first instance and the effect6s observed of replacing the 1g cystine with an equivalent quantity of methionine." (Note(s): #SM's estimated (normal, age 15y at BW = 60kg) daily requirement of Methionine + Cysteine is 20mg/kgBW/day, Approx 1200mg/day # "Rose found, however, that cystine in the diet has a marked sparing effect on methionine, reducing by 80-90% the minimal methionine needs of 3 (normal) subjects."(Rose & Wixom, 1955).) Nitrogen and sulfur intake were constant throughout the study. **Study Results**: When the supplementary cystine was replaced with methionine in SM's intake, he went into marked negative nitrogen balance, on reversal of the adjustment the nitrogen balance reverted, and on replacement of approx 70% of the supplementary methionine with cystine the nitrogen balance became markedly even more positive. The (CBS normal) control subject's responses to the same changes in intake were markedly nearly the exact reverse of SM's, though very roughly half an order of magnitude less. SM's plasma taurine approx 0.5mg/100ml roughly throughout but no data apparent for day20-26 Analogous (CBS normal) control data unavailable. SM's urinary taurine fell from 100mg/day (day1-14) to 10mg/day and remained there even through the mere reversion phase (day14-20-26) until the phase of replacement of approx 70% of the supplementary methionine with cystine (day26-37) when it was again 100mg/day at day 31 and 200mg/day at day 37. Analogous (CBS normal) control data unavailable. (continued 2 columns to left)

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(continued) Brenton DP et al (1966); Quart J Med; University College Hospital and Medical School, London, UK Note: The estimations of SM's daily requirement of methionine + cysteine (assuming normalcy of CBS, not the case) are derived from Shils et al (1999)	Case 2	RM, between 8-18months of age 3 admittances with severe vomiting, abdominal distension, and ileus…."In retrospect these episodes might have been due to mesenteric vascular thromboses.", recurrent respiratory infections (Respiratory Syncytial Virus isolated on one such), by age 15months behind on developmental milestones, at age 2.5y possible historic major epileptic seizure, liver palpable, mild skeletal abnormalities on Marfanoid continuum "The resemblance of his recent changes to those in acromegalic gigantism is striking….It is likely, therefore, that arachnodactyly will develop later.", ectopia lentis, severe mental retardation, "Grossly increased" platelet stickiness (continued from far rhs column) "…..After 5days on the diet ….Again he began to vomit, refused to eat or drink, and the diet was discontinued." ?: Was betaine used again this time?…could there have been a conditioned response from the pairing of this diet with the betaine the last time?…….. "Platelet stickiness was measured 3 times in RM. Once before starting the diet and on the 8th and 22nd day after starting it. On all three occasions the platelets were grossly abnormal in behaviour and the improvements induced by the diet in plasma amino acid concentrations were not obviously associated with any decrease in platelet stickiness….The dietary trial here was long enough to allow regeneration of all platelets, taking the life of a platelet to be 8days, so that failure to improve was not due to the persistence of permanently injured platelets in the circulation." ?: reduced methionine causes reduced SAM which then has less stimulation of any residual CBS activity which causes reduced cysteine which causes reduced sulfate….reduced heparin sulfation or some other cysteine-related dysfunction?……	Hcy-uria present, Equates to tHcy > 40uM	**Diet Protein/Methionine Study:** "…9.4g/day of natural protein was allowed in the diet in oatmeal porridge, potato, water-diluted double cream, Hienz tinned baby food, fruit, low-protein biscuits…..the diet was calculated to provide10-12mg/kgBW/day of methionine…." Note: RM's estimated (normal, age 18m at BW = 12.5kg) daily requirement of Methionine + Cysteine is 35mg/kgBW/day, Approx 440mg/day "….The essential amino acids (excluding methionine) were added to the food in the amounts recommended by Snyderman and her colleagues (Snyderman 1958) for babies together with histidine 35mg/kgBW/day, cystine 100mg/kgBW/day, and glycine 300mg/kgBW/day." "betaine hydrochloride tried by mouth for 10 days" (day20-30) **Study Results:** Pre-diet plasma Met: 12mg/100ml Diet plasma Met: 1-to-2mg/100ml Pre-diet fHcy = 130uM = tHcy 170uM Diet fHcy = 100uM =tHcy 140uM "The diet was tolerated fairly well during his admission, except for an occasional vomit in the evening. After his discharge home (day27) the vomiting increased in frequency and one week after the discharge the diet was discontinued….the vomiting ceased within several days of stopping the diet.…." ?:….betaine cerebroedema reaction??……. "….After 7 weeks on ordinary food at home the child was readmitted for another attempt at dietary treatment" After 5days: Pre-diet plasma Met: 18mg/100ml Diet plasma Met: 2.8mg/100ml Pre-diet fHcy = 180uM = tHcy 220uM Diet fHcy = 70uM =tHcy 110uM (continued 2 columns to left)

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Perry TL et al (1966); Pediatrics ; U British Columbia, Vancouver, Canada.	1 baby boy, CS, 3.5kg birthweight, product of a normal pregnancy and delivery, two elder siblings having homocystinuria such as to have warned in advance that this case might be subject to same. Note: To compare with 2 other untreated CBS-- siblings, see Perry et al (1968) below	Gains 500gBW in days 0-16 Gains no BW in days 17-29 Gains 280gBW in days 30-40 (Gains 600gBW….. ….continues….. ….. in days 41-69) "satisfactory" Note: Gaull GE (1967) provides a follow-up note on this case: "A recent letter from Perry indicates that at age 12m this patient weighed 10kg, could take a few steps alone, and used several recognizable words. The Griffiths Developmental Quotient was said to be 99. There were no abnormalities noted on physical examination, electroencephalogram, or X-ray of long bones. On a diet containing 27mg/kgBW/day of L-methionine he showed a fasting plasma methionine level of 6.2mg/dl and a total homocystine of 0.6mg/dl. They felt that they could not lower the plasma levels further without jeopardizing growth. This patient, in contrast to his two afflicted siblings, is developing normally despite elevated levels of methionine and homocysteine. Could this be because of normal cystine turnover during this critical first year of development? If we accept this limited data as evidence of a salutary effect, should we not consider whether it is due to the large supplement of cystine as well as to the limitation of methionine?" Note: Perry et al (1968) provide a follow-up note on this case: "..low-methionine diet and supplemental L-cystine constantly since the 16th day of life" now 3y old, "he has shown none of the clinical manifestations of homocystinuria."	Hcy-uria present, Equates to tHcy >,>> 40uM Ditto roughly throughout ,but various "nil" or "trace" readings for plasma fHcy and urinary Hcy seem dubious?..	Evaporated cow's milk formula Plasma Cystine assay unreliable Low-methionine formula of soy bean protein, corn oil, dextrimaltose, Cystine 100mg/kgBW/day additional supplement Plasma Cystine rises from 0 to 0.79mg/dl (normal newborn 0.7-2.0mg/dl) Breast Milk Cystine 100mg/kgBW/day additional supplement Plasma Cystine 0.32, 0.53mg/dl (normal newborn 0.7-2.0mg/dl) Low-methionine formula of soy bean protein, corn oil, dextrimaltose, Cystine 150mg/kgBW/day additional supplement Plasma Cystine 0.8, 0.52mg/dl (normal newborn 0.7-2.0mg/dl) Low-methionine formula of soy bean protein, corn oil, dextrimaltose, Cystine 200mg/kgBW/day additional supplement Plasma Cystine 0.92mg/dl (normal newborn 0.7-2.0mg/dl) Note: A 3-4m aged infant's daily requirement of methionine + cysteine is 58mg/kgBW/day Shils et al (1999), while that of a 2-5y aged child is 27, and that of a 10-12y aged child is 22 – roughly following this exponential trend back to the relevant periods of this case provides no quantification of requirements of any certainty, other than to say that it seems that the cystine supplementation could have been of roughly the right order of magnitude…and the plasma levels seem to support this…context of differences from normalcy in cysteine input to systemic circulation. ?: Some breast milk component required, at some stage?….. ?: does supplementary cysteine spare any cystathionine produced by any residual CBS activity?…..

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

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Komrower GM et al (1966); Arch Dis Childh; University College Hospital, London, UK.	1 Case	PW, female, the sixth offspring in a family with two previously diagnosed homocystinuric siblings "showing the classical signs" was able to be detected as homocystinuric and hypermethioninemic neonataly, and received thereafter a Diet: modified from that of the treatment for Maple syrup urine disease (Gelatin (deficient in branched-chain aa and methionine), milk, carrot, potato, lentils, cereal, fruit, Arachis oil, sucrose, mineral mix, essential amino acids, and cystine) "The plasma cysteine levels remained low at this time, so the intake was increased from 24 to 76 mg/kg. This had little effect. We wondered whether this might be due to poor absorption of cystine in the gut because of its low solubility, so, at 6months the more soluble calcium cystinate was given" Age Diet Cystine/CaCys Plasma Cystine Plasma Methionine Plas fHcy 1d 1.0mg/dl 0.8mg/dl ND 3d 1.2 1.5 7.2uM 9d 0.4 26.8 22 3w .081g/d 0g/d 0.6 0.4 2 5w 0.329 0 trace 0.7 ND 2m 0.329 0 0.3 0.7 ND 3m 0.329 0 0.8 0.4 trace 4m 0.329 0 0.6 0.6 0.02 6m 0.590 0 0.2 0.5 2 7m 0.590 0 0.2 1.7 2 8m 0.590 0 0.1 9.7 2 9m 0.590 0 0.2 1.6 6 11m 0.850 0.500 1.0 1.1 trace 13m 0.850 0.475 0.5 4.5 ND 15m 0.850 0.475 ND? 4.9 52 16m 0.850 0.475 ND? 13.2 18 16.5m 0.850 0.475 0.8 1.3 9 16.75m 0.850 0.475 1.9 0.2 ND 17m 0.850 0.475 0.7 0.7 ND 19m 0.850 0.475 1.5 0.5 ND 20m 0.850 0.475 1.2 0.7 2 22m 0.850 0.475 0.4 1.0 6 24m 0.850 0.475 0.3 1.2 ND 26m 0.850 0.475 0.2 0.8 11 Note: The anomalous rises in methionine and homocysteine around the 15-16m period appear to be the result of a friend of the family feeding the child extra-dietary foodstuffs. Outcome: at age 2.3y "is in excellent health, 11.8kgBW, height 86cm, walks, talks, feeds herself, IQ 97, EEG normal, normal discs and fundi, no sign of ectopia lentis, slight knock-knee, no radiographic abnormal bony changes, platelet stickiness 72% "considered well within the normal range of this technique." "Her mother is convinced that PW at this age is much brighter than were her affected but untreated sibs and is very like her normal sisters. Nevertheless, one must remember that several of the children reported on earlier were apparently normal for the first few years of life (Komrower and Wilson, 1963) and for this reason considerable reservations must be made in respect of the long-term prognosis." Note: Unfortunately more detailed comparisons with the siblings, if only brief, were not made here.	Hcy-uria present, Equates to tHcy > 40nM (roughly approx derivat'n) tHcy <40uM? 47uM 62 42 <40? <40? 40 40 42 42 42 46 40 <40? 92 58 49 <40? <40? <40? 42 46 <40? 51	See middle column
Gaull GE (1967); Amer J Dis Child; USA		See Perry TL et al (1966) above for his report on a communication received from Perry on the follow-up of that case.		

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Perry TL et al (1968); Lancet; U British Columbia, Vancouver, Canada.	CBS--Case 1	Patient A, female, at age 7y severely mentally retarded, ectopia lentis, significant Marfanoid continuum, repeated intravascular thromboses. Note: for use in comparison with treated-from-birth sibling CS detailed in Perry et al (1966) above "Her younger homocystinuric brother died at the age of 10m from bilateral renal vein thromboses and pulmonary embolism" (not a subject of this study). Patient B, male, treated-from-birth sibling of Patient A, see case CS in Perry et al (1966) above (not a subject this study)	Hcy-uria present, Equates to tHcy > 40nM	
	CBS--Case 2	Patient C, male, at age 16y, ectopia lentis, significant Marfanoid continuum, "During the year before he started the dietary treatment described below, he had 2 life-endangering episodes of cerebral thrombosis, which left him with a residual hemiplegia and further impairment of intelligence."	Hcy-uria present, Equates to tHcy > 40uM	
	CBS--Case 3	Patient D (sister of Patient C), female, at age 14y mild mental defect, ectopia lentis, some marfanoid continuum, no recognised intravascular thromboses. Dietary treatments: At some point in time each of Patients A, C, and D were commenced on a low-methionine diet "which provided approx 1g protein and 10mg of methionine per kgBW/day. Half of the daily protein and methionine intake was supplied by soybean protein ('Sobee', Mead Johnson). The remainder of the dietary protein and methionine was derived from a wide variety of cereals, vegetables and fruits. All foods of animal origin were excluded from the diet…..Each patient was also given daily supplements of dicalcium phosphate, ferrous sulfate, and a multivitamin preparation." Choline 10g/day was given intermittently, but a 100day period of it was centered about the time of the institution of the low-methionine diet in all cases; Patient A also received 10g/day for a 40day period 20days prior to the 100day period, and Patients C and D received also 10g/day for two periods 50days, 30days, after the 100day period. Pyridoxine (VitB6) 500mg/day was given for a 20day period separate from and partly occupying the intermission 'twixt the latter two choline periods in Patients C and D, and for 20day and 40day periods bracketing the choline periods but well separate from them in Patient A. (see also continuation next table, for cystine details) Results: The pyridoxine (VitB6) was not associated with any change in either plasma methionine or homocysteine in Patients C and D, so these seem to be VitB6-nonresponsive cases. In Patient A there was an association with lower plasma homocysteine paired with barely-visibly lower plasma methionine in the second period but not the first. The choline was fairly consistently associated with lower plasma homocysteine paired with higher plasma methionine, of lesser magnitudes during the low-methionine diet periods. The low-methionine diet was consistently associated with decreased plasma methionine and decreased plasma homocysteine. Note: The arrangement of these treatment periods was quite well done with a view to differentiating the effects, via maximising crossover in the context of ethical maintenance of the low-methionine diet once instituted. (continued below)	Hcy-uria present, Equates to tHcy > 40uM	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
(continued) Perry TL et al (1968); Lancet; U British Columbia, Vancouver, Canada.	(cont)	(continued) "Elimination from the diet of proteins relatively high in methionine content also eliminated much of the dietary cystine; and, since for the patient with homocystinuria, cysteine becomes and essential aminoacid, it was necessary to add cysteine or cystine to the diet. Thus each patient was given a calculated excess of L-cystine while on the low-methionine diet. The fact that the orally administerd L-cystine was absorbed, and was probably given in excess, was demonstrated by the regular presence of unusually large quantities of taurine, a metabolite of cysteine, on the patients' urinary aminoacid paper chromatograms. Despite the administration of L-cystine, plasma concentrations of cystine in our patients were always far below the normal range (30-50uM)….Tripling the dose of supplemental L-cystine did not produce an increase in plasma-cystine." Patient A's details only were provided, from which I excerpt (and assume that the values are for "free" ie fHcy etc and not "total" ie tHcy etc, and are therefore reduced disulfide forms as applicable) (values are mg/kgBW/day, g/day, g/day uM respectively): TreatDay DietMet SupCys Chol Plasma: MetO Hcy Hcy-Cys Cys 22 60 0 0 38 119 63 trace 110 60 0 10 201 15 38 13 124 60 0 10 117 7 18 14 208 60 0 10 130 85 44 4 240 15 1.5 10 20 48 44 6 268 10 1.5 10 trace 34 55 15 274 10 4.5 10 trace 20 46 20 286 10 4.5 0 trace 37 42 10 344 10 1.5 0 17 18 35 17 364 10 1.5 0 20 18 48 23 **Normal** **0** **0** **0** **30-50** To get tHcy equivalents double the Hcy value and add 40uM, then add the Hcy-Cys value to it, which gives a range of 75-340uM tHcy, mostly 100-180uM Note that the cystine values are not "far below the normal" and that if the cysteine from the mixed Hcy-Cys disulfide is added to the Cys, then quite often the sum is within the low normal region….. Note that the above facet possibly goes some way to explaining the failure of the tripling of the Cys supplement of raise plasma Cys. ?: Is Cys metabolized to taurine more quickly than normal?…	(derived) tHcy 340uM 108 72 254 180 163 126 156 111 124 **10, <30**	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
Carey MC et al (1968) ; Am J Med ; 3 Hospitals, Dublin, Ireland	9 CBS--	The following excerpts omit much detail that is generally in close agreement with information provided by other similar studies dealt with by me elsewhere iabove: " Ascertainment was by means of reviewing the records in our files of patients with presumed Marfan's Syndrome and/or ectopia lentis, using the cyanide-nitroprusside test; 5 patients were thus discovered. A further 2 were detected by screening the sibship of each proband, and one patient received treatment for coeliac disease for eighteen months before a diagnosis of ectopia lentis and then homocystinuria was made." Case MMcL normal platelet count, prothrombin time, activity and concentration, factor V, factor VII, clot retraction, clotting and bleeding times. Case PBu "seemed about half his age….a pitiful sight" (neglect?) "In 1960 he suffered a "green-stick" fracture of the right femur after a minor injury, in 1962 he fractured his left tibia and fibula in the midshaft and in 1966 fractures of 3 metacarpals were discovered after a fall. Union was slow and it was noted that there was undermineralisation of the long bones….crusted eczema on the chest wall" Case TK "Clinically the aortic bifurcation was occluded, and preoperative aortography confirmed this diagnosis. At op the internal aorta, both common iliac, common femoral and proximal profunda and superficial femoral systems were thrombosed with organised, friable, clot, leaving no plane of cleavage. ….. Autopsy: There was no macroscopic cross-ridging, a feature regularly described by previous investigators in homocystinuria. The liver weighed 910g and was congested; the diaphragmatic and anterior surfaces were mottled with fatty streaks…. the kidneys showed, besides fresh infarcts, recent thromboses in large arteries of arcuate size. Pads of fibrous tissue were seen on the intima of some large vessels. There was extensive centrilobular fatty change in the liver. Moderate myocardial fibrosis was found with intimal thickening of the coronary arteries; the anterior descending branch of the left coronary artery showed a fresh antemortem thrombus occluding the lumen; the coronary arteries also showed some "pads" such as were seen in the kidney. The lungs were congested, with a few small thrombi in the smallest vessels. The aorta showed considerable intimal fibrous thickening. A few foci of bluish cystic material were seen in the wall resembling medionecrosis but elastic tissue seemed regular in formation and amount." "Comments….Mandibular prognathism, which was seen in Cases 1, 2, 5 and 7, is a new addition to the protean manifestations of homocystinuria previously recorded….. The skin of the hands, elbows, knees and shins in Cases 1, 2, 3, 4 and 7 were covered with "tissue-paper" atrophic scars like those seen in porphyria and Ehlers-Danlos Syndrome…. Wound repair may also be poor due to an inadequate synthesis of cystine (cysteine? DV)…… At variance with previous reports, the unusual gait described as "shuffling", "ducklike" or "Chaplinesque" was not observed in our patients. However many were unable to climb stairs…. there were nonspecific EEG findings of diffuse cerebral dysfunction in all patients…… Only 1 patient in our series, a girl (Case 9), had thromboembolic features. The presumed "encephalitis" in 1961 and "meningitis" in 1963 in this patient were probably due to recurrent cerebral venous thrombosis."
Parkinson MS, Harper JR (1969); Proc Roy Soc Med; General Hospital, Northamp-ton, UK?	1 CBS-- general comm't	"JD, boy aged 11.....classical features.....marked emotional lability.....plasma Hcy(ine) 185uM…. Unfortunately, dietary treatment in our patient was not successful. The unpalatability of the food regimen soon led to dietary indiscretions. The need for regular hospital attendances for plasma and urine monitoring became unacceptable to both patient and parents, and blood sampling was eventually refused. The scheme had to be abandoned after four weeks……While the value of dietary therapy in the affected newborn is not in dispute, the benefits for the adolescent would not seem clear. The added practical difficulties of dietary restriction in these mentally retarded children are considerable, while from the humanitarian point of view, this dietary policy seems hard to justify." Note(s): Methionine restriction needs to be better differentiated from cysteine supplementation, as the former addresses Hcy levels, while the latter addresses amino acid (and taurine, sulfate?) requirements, and the former requires actual dietary modification while the latter is supplemental (ie pill/drink form). Methionine restriction needs to be better differentiated from its monitoring ie via blood sampling. Blood sampling needs to be better differentiated from urine sampling re invasiveness and patient convenience. VitB6-responsivity requires addressing. Age of implementation is a factor in sustainability, particularly of diet, as well as being crucial to outcome.

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Hopkins I et al (1969); J Pediatr; Royal Children's Hospital, Melbourne, Australia.	3 CBS--, siblings	"Our patient, a 17month old girl with delay in acheiving the normal developmental milestones and some enlargement of the head, first noted 6months before admission, was admitted with 24hour history of fever, vomiting, and right-sided convulsions followed by a left hemiplegia. On admission signs of raised intracranial pressure were noted. Right carotid angiography, pneumoencephalogram, and electroencephalogram were not diagnostic and the cerebrospinal fluid contained 18 RBCs per uml and protein 10mg%. Repeat lumbar puncture following deterioration showed xanthochromic cerebrospinal fluid with a pressure of 255mmH_20. She subsequently developed decerebrate rigidity and died 11 days after admission. At autopsy, organised thrombus of all the dural sinuses, old infarction of the thalamus, and calcified massive thrombus of the pulmonary artery were found. Subsequently two siblings, one of whom suffered an intracranial thrombosis but has survived, were found to have homocystinuria. We feel that angiography is contraindicated in homocystinuria; our patient did deteriorate following the procedure. Thus, in any child suspected of intracranial thrombosis, before proceeding to angiography one should test for homocystinuria. Screening of the siblings…."		
McCully KS (1969); Am J Pathol; Massach'ts General Hospital, Boston, USA	1 Case	(presumptive CBS--) retarded mental development, congenital dislocation of the lenses, death from vascular thrombosis in childhood at age 8y both carotid artery walls thickened with severe narrowing of the lumens, right carotid artery further narrowed to almost complete occlusion by organised thrombus, right half of circle of Willis containing a recent thrombus, right cerebral hemisphere softened and pale "result of an extensive early infarction", no gross evidence of disease within the heart, aorta, pulmonary artery, venae cavae, or other major vessels, except for the carotid arteries, right internal carotid artery intima thickened by a marked proliferation of loose fibrous connective tissue, disorganization of the media, and reduplication, fraying, thickening, and discontinuity of elastic fibres of the internal elastic membrane, a toluidine blue stain showed small amounts of metachromatic ground substance within the media and intimal fibrous tissue, "widespread focal alterations in the medium-sized and small arteries of the thymus, adrenal, kidney, heart and lymph nodes. In many medium-sized arteries there was focal narrowing of the lumen by intimal fibrosis, associated with splitting, irregularity, and focal discontinuity of the internal elastic membrane. As exemplified by the findings in the kidney and adrenal glands, many small arteries and arterioles were surrounded by a moderate proliferation of fibrous tissue containing increased numbers of fibroblasts, collagen fibres, and thin, irregular elastic fibres; the media of some of these vessels was moderately thickened by hyperplastic smooth-muscle cells. In the kidney many of the glomeruli were slightly to moderately hypercellular because of increased numbers of mesangial and endothelial cells; marked red cell congestion was present in many of the glomerular capillary tufts. One section of the aorta was normal. Moderate centrilobar fatty change was present in liver parenchymal cells; and extensive acute bronchopneumonia was found		

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
McCully KS (1970); Am J Pathol; Massach'ts General Hospital, Boston, USA	2 CBS-- cases, And, 2 Control , human Cell lines	"Fragments of dermis were obtained from a skin biopsy of a 9y-old, mentally retarded female with documented cystathionine synthetase deficiency and homocystinuria. A serially cultivated cell line, 49 HC, was obtained as an outgrowth of cells from the dermal fragments…A second cell line 21HC, obtained from a 27y-old male with homocystinuria, and 2 normal cell lines, 26JL and 120JL, obtained from a 3y-old female and a 20y-old male were cultivated in the same way" ("…both methionine and cystine, found in small amounts in the cultured monolayer, are both present in adequate concentrations in the culture medium.") "Results: Cells from the CBS—consistently produced moderate amounts of extra-cellular and intracellular material which formed aggregates and granules of varying size, frequently aligned along cellular processes and extracellular appearing fibrils but also scattered between the cells. The material was light red when stained with Giemsa but metachromatic red-violet when stained with toluidine blue. Reduced numbers of fibrils of normal morphology were present either in confluent areas or in sparsely populated areas. The abnormal material was produced throughout the growth cycle. When Hcy or Hcy thiolactone was added to the culture medium, larger amounts of the abnormal substance formed. In addition, comparison with control cultures revealed that the total number of cells in the monolayer was decreased after 1 week's growth when Hcy 1000uM was added to the medium. The addition of Hcy 200-2000uM to the medium did not, however alter the cloning efficiency (26%) of the CBS—cells. In cultures with Hcy in the medium, some of the cells were found to contain small, uniform sized cytoplasmic granules, less than 1u in diameter, frequently distributed in a short linear array at the margin of the cytoplasm, and extending along extracellular appearing fibrils. Cells from the normal patients formed large numbers of long extracellular and intracellular fibrils of varying length and diameter without evidence of granular aggregated material. Addition of Hcy or Hcy thiolactone (He doesn't give conc…apparently 1000uM from his Figure caption.) to the medium, however, resulted in the formation of moderate amounts of light red-staining granular flocculent substance and small amounts of fine granular material associated with fibrils. …… Examination of the confluent layer of normal cells, by polarized light, revealed numerous, long, moderately birefringent fibrils. However, only small numbers of short, irregular, clumped birefringent fibrils were observed in the confluent monolayer of CBS—cells." "Discussion and Conclusions: The altered solubility of the glycoprotein and proteoglycan fractions isolated from the aorta of a patient with homocystinuria (cites Carson NAJ et al 1966) is probably the result of an altered state of aggregation and molecular conformation of proteoglycans synthesized by aortic cells. The ectopia lentis commonly observed in the syndrome was found to result from replacement of the normal fibrillar structure of the zonular fibers by irregular, granular material (cites Henkind and Ashton 1965). This morphologic change is very similar to the replacement of fibrils by granular fragmented material observed in the cultured cells. The findings of increased amounts of soluble collagen and an increase in the amount of noncross-linked collagen in two individuals with homocystinuria (cites Harris and Sjoerdsma 1966( )), as well as some features of the various connective tissue abnormalities in the syndrome, are presumably due to partial inhibition of normal collagen cross-linking by abnormal proteoglycans elaborated by the CBS—cells of connective tissues…..The glycousaminoglycans of human skin cultures do however, contain appreciable amounts of esterified sulfate. If increased homocysteine concentrations within the cell produced a change in the number or distribution of esterified sulfate groups on the carbohydrate side chains of the proteoglycan molecules synthesized by the cell, a drastic change in the conformationof the macromolecule at physiologic pH might be expected to result from the consequent alteration in the ratio of charge on polypeptide core to charge on surrounding carbohydrate envelope…..The finding that the addition of Hcy (1000uM from figure caption) to the culture medium of normal cells produced proteoglycan abnormalities similar to those found in CBS—cell cultures suggests that elevated concentrations of exogenous homocysteine are capable of producing arteriosclerotic and connective tissue changes in vivo in normal individuals without enzyme deficiencies."

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
McCully KS (1972); Am J Pathol; Massach'ts General Hospital, Boston, USA	2 CBS—cases, And, 2 Control human Cell lines	"Fragments of dermis were obtained from a skin biopsy of a 9y-old, mentally retarded female with documented cystathionine synthetase deficiency and homocystinuria. A serially cultivated cell line, 49 HC, was obtained as an outgrowth of cells from the dermal fragments…A second cell line 21HC, obtained from a 27y-old male with homocystinuria, and 2 normal cell lines, 26JL and 120JL, obtained from a 3y-old female and a 20y-old male were cultivated in the same way" "When pyridoxine was added to parallel cultures of the 21HC cell line the growth rate of the cells and production of protein was greatly increased, but there was no effect of added pyridoxine on the 49HC or the 120JL. The effect of pyridoxine correlates with the clinical (VitB6-responsivity: 21HC, yes; 49HC, no) " "To determine ^{35}S binding, equal numbers of cells were passaged into Eagle's minimal assential medium (MEM) with 10% fetal calf serum, containing $^{35}SO_4^{2-}$ (New England Nuclear), 1uCi/ml and 0.8mM, or ^{35}S-Homocysteine thiolactone (Amersham), 1uCi/ml and 1.0mM, and refed twice weekly. The sulfate concentration of some media was doubled by adding 0.2g/l of $MgSO_4.7H_20$. The two concentrations of sulfate used, 0.8 and 1.6mM are both within the normal physiological range found in plasma. After the cultures reached confluence (2-8weeks), the medium was removed, and the monolayer was rinsed twice with cold phosphate-buffered saline (PBS), pH 7.0, and scraped from the dish into fresh PBS. The mixture was homogenized thoroughly in a glass homogenizer, and aliquots were taken for counting by liquid scintillation, using Bray's solution, and for protein determination, using the Lowry method." **Important Note**: The procedure **precludes differentiation of extracellular phenomena from intracellular phenomena**. **Results:** Initially: "The intercellular orientation and distribution of the CBS—cells over the surface of the culture dish was abnormal, since areas of sparse growth alternated with areas of growth in which cells formed multiple layers containing mitotic figures." The VitB6-responsive CBS—cell line was purportedly more abnormal than the VitB6-nonresponsive CBS—cell line, unexpectedly. Abnormality correlated with binding of as ^{35}S as $^{35}SO_4^{2-}$. The abnormal granular substance was both **within and between** (see Important Note immediately above) the CBS-- cells. After some hours of puzzling over the inexplicably and apparently deliberately severely encrypted tabulation of results presented by KMcC, the following **two** tabulations (Series 1 and Series 2) are what I think is what his results presented in Table 1 were (They appear together below due to the need to view them concurrently):

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Authors, Journal, Site	Study Group	Disease
(continued) McCully KS (1972); Am J Pathol; Massach'ts General Hospital, Boston, USA	(cont) 2 CBS--ases, And, 2 Control human Cell lines	

Series 1:

Cells	$^{35}SO_4^{2-}$	SO_4^{2-}	Hcy'one	$^{35}SO_4^{2-}$ nmol/mgProt
Cont1	800uM	0	0	14, 16
Cont1	800uM	800uM	0	20, 19
Cont1	800uM	0	1000uM	9.3, 6.3, 9.3
Cont2	800uM	0	0	7.8, 8.1, 9.1, 9.4
Cont2	800uM	800uM	0	18, 20, 30, 26
Cont2	800uM	0	1000uM	7.0, 7.2, 8.6, 8.2
CBS--1	800uM	0	0	29, 26, 33
CBS--1	800uM	800uM	0	58, 62, 64
CBS--1	800uM	800uM	1000uM	88, 98, 112
CBS--2	800uM	0	0	79, 67
CBS--2	800uM	0	1000uM	84, 62, 85

Interpretation (verbalisation):

#1 From Series 1, homogenates form the CBS--individuals, grown to confluence in medium containing $^{35}SO_4^{2-}$, had more bound sulfate per mg protein than homogenates from the normal individuals.

#2 From Series 1, when the concentration of unlabelled sulfate was increased in the medium, all the cell lines homogenates had more protein-bound $^{35}SO_4^{2-}$. This is unexpected on the basis of the assumption of the simplest molecular arrangement type, where a reduction in protein-bound $^{35}SO_4^{2-}$ would rather have been expected, and complicates the other interpretations.

#3 From Series 1, adding unlabelled homocysteine thiolactone to the medium of the CBS--, with the total sulfate kept constant (at 800uM) was associated with a slight reduction of the amount of sulfate bound to protein for the normal controls, but with a slight increase of the amount of sulfate bound to protein for the CBS--, both magnitudes being small relative to the levels.

#2&3 We note that the addition of Hcy to CBS-- cultures has the same association direction of an increased binding of sulfate, as adding extra (unlabelled) sulfate, while the addition of Hcy to the normal control cultures to the contrary is associated with rather a decreased binding of sulfate

Series 2:

Cells	SO_4^{2-}	^{35}S-Hcy'lactone	Hcy'one	^{35}S-Hcy'onenmol/mgProt
Cont1	800uM	1000uM	0	44, 69, 49
Cont1	1600uM	1000uM	0	41, 51, 54
Cont2	800uM	1000uM	0	55,69
Cont2	1600uM	1000uM	0	104, 94, 54, 85
CBS--1	800uM	1000uM	0	31, 51, 50
CBS--1	1600uM	1000uM	0	54
CBS--2	800uM	1000uM	0	42, 24, 38
CBS--2	1600uM	1000uM	0	40

Verbalisation & cautioned Interpretation (In conjunction with Series 1):

Control protein binds Hcy in preference over sulfate, while CBS-- protein to the contrary binds sulfate in preference over Hcy.
The meaning of this might be that:
in the CBS-- cells there is a deficit of endogenous sulfate due to the defective transulfuration pathway, which the cell attempts to redress by incorporating more exogenous sulfate,
and that endogenous Hcy has already bound to a large proportion of binding sites in the CBS-- cells, leaving less opportunity for exogenous Hcy to bind,
However, as we are **unable to differentiate extracellular phenomena from intracellular phenomena**, such interpretations must be viewed with caution to say the least.

No consistent effect of adding extra sulfate to medium on the binding of exogenous Hcy

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Authors, Journal, Site	Study Group	Disease
(continued) McKully KS (1972); Am J Pathol; Massach'ts General Hospital, Boston, USA	(cont) 2 CBS-- cases, And, 2 Control human Cell lines	"Aliquots of some homogenates were mixed with cold 5% trichloroacetic acid (TCA) and filtered through Millipore filters; the filters and filtrates were counted in Bray's solution." Results: Cells Precursor % ^{35}S TCA-Insoluble nmole35S/mgProtein Cont2 $^{35}SO_4^{2-}$ 8 1.2 Cont2 ^{35}S-Hcy'lactone 28 15.1 CBS--2 $^{35}SO_4^{2-}$ 17 12.4 CBS--2 ^{35}S-Hcy'lactone 33 11.4 CBS--1 $^{35}SO_4^{2-}$ 12 3.5 Interpretation: Note: All ^{35}S-Hcy'lactone is exogenous, while some $^{35}SO_4^{2-}$ will be endogenous from the processing of ^{35}S-Hcy'lactone down the transulfuration pathway, mostly in the normal cells were it is available - "The observation that CBS—cell monolayers bind ^{35}S in an amount aprox equal to or lower than that of normal cell monolayers may be explained by assuming that cellular synthesis of unlabelled homocysteine and homocysteic acid derived from methionine was increased due to CBS-- inability to convert the Hcy to cystathionine (>>>sulfate). The increased amount of unlabeled Hcy derived thusly would be expected to……" See Overall Interpretation below "Aliquots of some homogenates were exhaustively dialyzed for 24hours against cold calcium- and magnesium-free buffer containing EDTA 0.2g/l and K_2SO_4 0.28g/l, and the radioactivity remaining within the dialysis bag was determined." Results: Cells Precursor %^{35}S Nondialyzable nmole35S/mgProtein CBS--1 $^{35}SO_4^{2-}$ 7.5 2.2 CBS--1 ^{35}S-Hcy'lactone 35 15.4 Interpretation: See Overall Interpretation below "Homogenates of cells cultured in ^{35}S-Hcy thiolactone were dialyzed against buffer containing EDTA and K_2SO_4, hydrolysed with 6 M HCl at 110C for 22hours under N2 and chromatographed on sulfonated polystyrene resin." Results: The CBS--1 assay recovered 88% of ^{35}S as $^{35}SO_4^{2-}$, while the Dunn-4 control recovered only 73% of ^{35}S as $^{35}SO_4^{2-}$. Both lines were approx equivalent for methionine sulfoxide, methionine, Hcy, Hcy(ine), and Hcy thiolactone. And conversely to the former, the CBS--1 assay recovered only 2.4% of ^{35}S as Unidentified, while the Dunn-4 control recovered 15% of ^{35}S as Unidentified. Interpretation: See Overall Interpretation below **Overall Interpretation:** As we are **unable to differentiate extracellular phenomena from intracellular phenomena,** the most that I am prepared to venture is that CBS-- skin cells produce an abnormal proteoglycan that is granular instead of fibrillar, and are sulfate deficient. KMcC ventures various surmise additional to this but due to the major limitation noted, which he does not sufficiently address in surmise, I will not reproduce and discuss them further here – the methods and data have been provided in sufficient detail to enable readers to form their own surmise – and they may turn to the original if they disagree with my conclusion or think it too incomplete.

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level
Cross HE, Jensen AD; (1973) Am J Ophth; Johns Hopkins Hospital, Baltimore, Maryland, USA.	CBS-- Vs Fibrillin gene Marfan Syndr	"The triad of dislocated lenses, skeletal anomalies, and cardiovascular disease may be present in both the Marfan Syndrome and homocystinuria….. The cardiovascular complications in homocystinuria are secondary to thrombosis and occlusion, mainly in medium-sized arteries and veins, in contrast to the defects in the valves and aorta often found in the Marfan Syndrome. Cerebrovascular thromboses, myocardial infarction, pulmonary emboli, intermittent claudication, and even death at a relatively young age often result. …Ectopia lentis, cataracts, myopia, optic atrophy, retinal detachments, and secondary glaucoma occur in both disorders. ... McKusick suggested that the lenses are more likely to be displaced downwards in homocystinuria than in the Marfan Syndrome, but quantitative comparisons are lacking." "It is apparent that ocular abnormalities are prominent features of both the Marfan Syndrome and of homocystinuria. The most frequent manifestation is, of course, ectopia lentis, but retinal detachment is the most serious threat to vision in both disorders." "Records of the Wilmer Institute, the division of medical genetics, and the central records library of the Johns Hopkins Hospital were searched for all patients with the Marfan Syndrome and homocystinuria":	
	42 CBS--Homocystinuria, ascertained by amino acid chromatography on ectopia lentis cases referred from ophthalmologists.	"Among 42 patients with homocystinuria, 38 (90%) had dislocated lenses (note ascertainment…). Dislocations were remarkably symmetrical, and no individual with unilateral dislocation was found. Visual acuity was normal in all eyes with lenses in the normal position. Ectopia lentis was discovered by the age of 5years in approximately 38% of homocystinuria patients and never after 25years of age. Only 1/3 escaped detection until age>10years. 1/3 of these lenses were dislocated inferiorly and 49% were displaced nasally. No lenses were dislocated inferotemporally or directly superiorly and only 4% had any temporal dislocation and only 4% had any temporal dislocation. 19% migrated nto the anterior chamber and 14% were found lying in the vitreous."	Hcy-uria present, Equates to tHcy > 40nM
	142 Marfan Syndrome, all without homocystinuria, ascertained by clinical evaluation, and thereafter unselected.	"A dislocated lens was detected in 79% of eyes in the Marfan Syndrome. In all except 3 patients the dislocation was bilateral….In patients with the Marfan Syndrome, the lense may dislocate in any direction, but most frequently moves upward. Fully 68% of lenses were displaced either superiorly, superonasally, or superotemporally. The direction of horizontal displacement is more nearly random…..Luxation into the posterior chamber is unusual, and occurred in only 3% of eyes with ectopia lentis. Curiously, no patient with the Marfan syndrome was seen with the lens in the anterior chamber, although atotal fo five lenses subsequently migrated there, at an average age of 20years." "Anomalies of the iridocorneal angle such as abnormal iris insertions with bridging pectinate strands, inconspicuous Schwalbe's line, wide iris processes, and a broad trabecular meshwork may be unique features of the Marfan Syndrome, since they have not been reported in homocystinuria, but the statistical documentation necessary for any diagnostic application of this distinction is not available."	Approx normal?

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Harker LA et al (1974); NEJM ; U Washington Sch Med, USA.	4* CBS--Cases	Case 1: Female, at age 20y homocystinuria detected, by which time at least one episode of thrombophlebitis and pulmonary embolism, at age 24y IQ 50, moderate Marfanoid continuum, calcification of aortic arch, occluded left carotid artery, flow reversal in left supraorbital artery, diminished popliteal flow bilaterally, Q waves V5 and V6 of ECG	"245uM Hcy(ine)" Probably "fHcy", = 300uM tHcy, otherwise literally = 530uM tHcy "179uM Hcy(ine)" Probably "fHcy", = 220uM tHcy, otherwise literally = 400uM tHcy	Nil After 14d of VitB6 350mg/ day
		Case 2: Female, at age 8y homocystinuria detected, at age 13y IQ 70, bilateral ectopia lentis, decreased flow in left carotid and both superficial femoral arteries	"168uM Hcy(ine)" Probably "fHcy", = 210uM tHcy, otherwise literally = 380uM tHcy "33uM Hcy(ine)" Probably "fHcy", = 73uM tHcy, otherwise literally = 106uM tHcy	Nil VitB6 50mg/d
		Case 3: Male, at age 16y homocystinuria detected, ectopia lentis, probable history of pulmonary embolism at? age 19y mentally deficient, malar flush, moderate Marfanoid continuum, non-specific ST-segment changes on ECG, decreased flow in right superficial femoral and left popliteal arteries	"202uM Hcy(ine)" Probably "fHcy", = 240uM tHcy, otherwise literally = 440uM tHcy "5uM Hcy(ine)" Probably "fHcy", = 45uM tHcy, otherwise literally = 50uM tHcy	Nil VitB6 300mg/d
		Case 4: Male, at age 16y homocystinuria detected, mild mental deficiency, ectopia lentis, two pulmonary embolizations with occlusions of right, and left, femoral veins, acute spontaneous occlusion of the right superficial femoral and popliteal arteries eventually requiring below-the-knee amputation,	Not given "no drop"	Nil VitB6 300mg/d
	4* CBS--,	Platelet Studies (1): Count Survival Cases 1&4 (VitB6-nonresponsive) 297,000/ml 5.0days Cases 2&3 (VitB6-responsive) 197,000/ml 8.6days	"40uM Hcy(ine)" Probably "fHcy", = 80uM tHcy, "140uM Hcy(ine)" Probably "fHcy", = 180uM tHcy,	Below as noted above…
	35 normal control	35 normal controls 250,000/ml 9.5days	"0uM Hcy(ine)" Probably "fHcy", = <<40uM tHcy,	
(Continues)		The analogous fibrinogen and plasminogen data was less remarkable in magnitude but corroborative inasmuch as VitB6-nonresponsives fibrinogen and plasminogen both had reduced survival vs the approx = VitB6-responsives and normal controls.	(Continues)	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
(Continued) Harker LA et al (1974); NEJM ; U Washington Sch Med, USA.	(Cont)	(Continued) "Patients unresponsive to VitB6 (Cases 1 & 4) were restudied while receiving a combination of dipyridamole, 100mg, and 1g of acetylsalicylic acid once a day; platelet survivals were prolonged to 8.3 and 8.7days respectively. These patients have now been maintained continuously7 on this combination for the past 3years, without any clinical evidence of vascular occlusive or thromboembolic events, as compared with a minimum of 5 thrombotic episodes in the year before therapy." Platelet Studies (2): Bleeding Time / Collagen Fiber Adhesion / GlassBead Retention / Phospholipid Availability / Clot Retraction		
	4* CBS--Cases	4.5min 48%adh 40%ret 67sec 48%of clot	"90uM Hcy(ine)" Probably "fHcy", = 130uM tHcy, (rough deriv'n, simple average)	Below as noted above…
	35 normal control	4.2min 51%adh 42%ret 65sec 47%of clot **Their Discussion** (part of): "Platelet utilization is closely related to homocysteinemia as evidenced by the inverse first-order relation between platelet survival and Hcy concentration in both patients and baboons. Platelet destruction, however, is not a direct toxic effect of Hcy on platelets, since dipyridamole (platelet inhibitor) therapy blocks platelet consumption without decreasing the concentration of homocysteine in the plasma. Furthermore, we found no evidence to support the notion that the thrombotic predisposition in homocystine ("Hcy-uria"?…) might reflect increased platelet utilization secondary to enhancing platelet reactivity. Platelet-function tests gave normal results and remained unchanged in both patients and animals. In view of the similarities of the thrombotic process of prosthetic and arterial embolic disorders, it was concluded that the underlying process of homocystinemic thrombosis probably involves formation of platelet thrombus on altered, nonendothelialized endarterial surfaces. This formulation was further supported by the known atherosclerotic vascular changes characteristic of homocystinuria"	"0uM Hcy(ine)" Probably "fHcy", = <<40uM tHcy,	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
Poole et al (1975) J Clin Investig	6 Vit B6-responsive CBS—And 5 Vit B6-nonresponsive CBS--	(from abstract) "Apparent nitrogen balances and urinary sulfur excretions were described for normal subjects, seven cystathionine synthase-deficient patients, and a single cystathioninuric patient on semisynthetic diets containing low-adequate amounts of methionine and very low amounts (12 mg daily, or less) of cysteine. The amounts of supplemental cysteine required to prevent abnormally high nitrogen or sulfur losses were determined. The five cystathionine synthase-deficient patients who had low residual activities of this enzyme detected in fibroblast and/or liver extracts did not lose more nitrogen or sulfur on diets virtually devoid of cysteine than did the normal subjects. These results suggest that the widely expressed opinion that cysteine is an essential amino acid for cystathionine synthase-deficient patients requires modification." (from body of text) "All of these observations together suggest that 310-510 mg cysteine is adequate for patients with virtually no residual cystathionine synthase activity detected by present methods." "The hepatic cystathionine synthase activities of the B6-responsive patients in their basal states (i.e. on B6 intakes provided by normal diets only) were 1-2% of the mean control value (19, 49). Thus, it appears that a residual activity of 1-2% of this enzyme endows the patient with the capacity to convert at least 20-50% of the normal methionine intake to cysteine.." My comments: In table IV, subject Jo. Ho. (one of the vitamin B6-responsive CBS—patients) has nitrogen balance results in the opposite direction of that expected on the low versus high cysteine intakes – with only 7 subjects in the study, this result, together with other variations from the average result, throws a fair bit of doubt on the whole study. Also, the statistical significances of the difference observed were not provided, and are in doubt due to the variations from the average noted just above. Moreover, the result does not argue strongly against the benefit of cysteine supplementation for all CBS—pateines, Vit B6-responsive and –nonresponsive, because some proteins have a higher content of cysteine than others, and the fact that in this experiment with only 7 CBS—subjects, over a a very short period of time, were only roughly close to being in nitrogen balance, does not argue strongly that over the whole of a lifetime, a CBS—patient of either B6 type without supplemental cysteine would be fairly likely to evolve by middle or old age significant disease due to the lack of that supplemental cysteine. Aside from cysteine in body proteins, there are also other metabolites of cysteine that are potentially of importance – cystathionine, glutationine and its anti-oxidant activity, sulphate, and hydrogen sulphide, just for examples. Many chronic diseases are known to have the contributing factors present for many years before the diseases finally develop to be clinically manifest in middle or old age.

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Murdoch JC et al (1977) ; BMJ ; Lennox Castle Institution, Glasgow, Scotland, UK.	CBS +++ trisomy 70 Downs Syndr	# 70 Down's Syndrome patients had ss or nearly ss lower systolic and diastolic blood pressures (consistent across age and sex dichotomisation subsets) than 70 age- and sex-matched non-DS mental defective patients living in the same institution # These DS patients and non-DS mental defective patients had remarkably similar cholesterol and triglycerides to each other. # Postmortem examination of 5 of these DS patients aged 40-66y showed a complete absence of atheroma, whereas a similar number of non-DS mental defective patients aged 42-80y were found to have mild or severe atheroma. # 123 normal community controls without family vascular disease history aged 20-65 had ss or nearly ss cholesterol and triglycerides than the abovenoted DS and non-DS institutionalized mental def's ?: Do **various** metabolic derangements cause atheromatous dis?…

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Almgren B et al (1978); Acta Chir Scand; U Hospital, Uppsala, Sweden.	1 CBS-- age 35y	" Man, 35, admitted January 1977 for evaluation of increasing right calf claudication since the early 1970s. At admission he had a walking distance of only 100metres. There were no known cardiovascular cases among his relatives. He had been smoking 20 cigarettes/day since age 20. Bilateral extirpation of his eye lenses had been performed in 1971 because of luxation. The patient appeared older than 35years. He had a course, light-coloured hair. He had a normal body build and there was no sign of mental retardation. Heart, lung and abdomen were normal at physical examination. ECG and laboratory tests including blood lipids were also normal. Arteriography demonstrated occlusion of the right superficial femoral artery. A femoro-popliteal vein bypass was performed. The operation was uncomplicated and the function of the leg was restored to normal. In September 1977, the patient was readmitted for further investigation. All routine tests were within normal limits. …. The cyanide-nitroprusside reaction of urine was positive…. Treatment with pyridoxine 320mg/day was started. Subsequent homocystine tests in urine were negative, indicating a favourable response to treatment. On January 8 1978, he noted the sudden onset of sever abdominal pain. On admission a pulsating abdominal mass was found. Aortography showed a large abdominal aortic aneurysm. In retrospect this aneurysm could be seen in the previous arteriograms. Emergency laparotomy was performed and a large leaking aneurysm was found with retroperitoneal bleeding. The aneurysm measured 10*13cm and large organised thrombi covered the inner aneurysmal wall. The aneurysm was resected and a bifurcated dacron graft was inserted into the common iliac arteries. The postoperative course was uneventful. Microscopic examination of the aneurysm showed advanced atherosclerotic degenerative changes."	Hcy-uria present, Equates to tHcy > 40uM Hcy-uria no longer present, Equates to tHcy < 40uM	Nil VitB6 320mg/d

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Grobe H et al (1979); Ped Res ; U Munster, Germany (is an abstract only in the journal)	3*CBS--VitB6-responsives	"Collagen-, ADP- and adrenalin-induced platelet aggregation was decreased (not my misprint) before treatment." Platelet factor 3 availability reduced, Open canalicular system occupancy 7.2% of total platelets area, Alpha-granules 7.2%	Higher: Hcy-uria present, Equates to tHcy > 40uM	Nil
		"Collagen-, ADP- and adrenalin-induced platelet aggregation was returned to normal when homocystine disappeared from plasma." Platelet factor 3 availability returned to normal, Open canalicular system occupancy 4.5% of total platelets area, Alpha-granules 11.1%	Lower: Hcy-uria not present, Equates to tHcy < 40uM	Unknown but was some – VitB6? + ?……
	5*CBS--VitB6?non/responsives already under treatment	"Functional and morphological studies in the 5 patients already under treatment showed normal results." **Their Conclusion**: We conclude that the platelet alterations in untreated patients result from their refractory stage after a release reaction has already taken place; the latter may be caused by the Hcy-induced endothelial lesions. In adequately treated patients, our data suggest that no additional administration of inhibitors of platelet aggregation is necessary."	Unknown, assume successfully?..	Unknown but was some – VitB6? + ?……
Hill-Zobel RL et al (1982); NEJM; Johns Hopkins U, Baltimore, Maryland, USA.		Platelets from 50ml of venous blood were harvested and labelled with ^{111}Indium in an aseptic open test tube system. All patients and controls had platelet counts within the normal range. Then reinfusion, and blood samples obtained at 90min and daily thereafter for 10days. Platelet Survival Times according to 3 different mathematic'l models: Linear Model / Exponential Model / Multiple-Hit Model		
	6*CBS--VitB6-responsives	9.33days / 3.19days / 8.02days	Hcy-uria not present, Equates to tHcy < 40uM	VitB6 500mg/d, Folate 10mg/d
	6*CBS--VitB6-non responsives	9.51days / 3.33days / 7.94days Inset: "The diet during the study was selected by the patients from an unmodified regular hospital menu. Daily calorie, protein, methionine, and cystine intake were determined by a dietician; the average values for all patients over the 12day study were 1215-3160kcal, 45-150gprotein, 1050-4000mgMethionine, and 700-2000mgCysteine per day" Note: Here, I take it that "determined" means only "calculated", and not "prescribed", and that no supplemental cysteine was used, ostensibly…	"48-118uM Hcy(ine)" maybe "fHcy", = 88-158uM tHcy, otherwise literally = 136-276uM tHcy, suspect the latter?...	No VitB6, No folate, Methio-nine abnorm'y high, Cysteine abnorm'y low And see "Inset"
	11*normal volunteers	9.81days / 3.25days / 8.40days Additional Result: Spleen ^{111}Indium accumulation at 1day CBS--50%dose , Controls 40%dose; at 3days all subjects 50%dose, and, Liver ^{111}Indium accumulat'n from 90min-3days all subjects 15%dose	Hcy-uria not present, Equates to tHcy < 40uM	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatment and biochem etc contexts
Wilcken DEL et al (1983); NEJM ; Prince Henry Hospital, Sydney, Australia.		Methionine+/-SD Cysteine ("fCys"?)+/-SD	"fHcy"?…+/-SD.	
	10 CBS--: (by enzyme, Hcy-uria, high methionine), aged 6-37y, (8 VitB6-nonresponsives, 2 VitB6-partially responsives).	330uM+/-347 56uM+/-15	146uM+/-61 =186uM tHcy?..	VitB6>=100mg/d Folate 5mg/day, 1 patient on diet <17mgMethionine/ kgBW/day, 5 patients on diets low-protein
		P<.01 P<.001	P<.001	
		592uM+/-396 83uM+/-12	34uM+/-20 = 74uM tHcy?....	(As above, but with Betaine2*3g/day in orange juice)*(5-13months
	25 Controls: adults, no other details.	26uM+/-7 131uM+/-34	4uM+/-1 = <40uM tHcy?...	No details
		"There were striking clinical changes after betaine treatment in some patients. In 2 there was a pronounced darkening of the hair, which was previously fair. Parents or schoolteachers gave unsolicited reports of improvement in the behaviours of 5 patients, although formal testing could not be undertaken to substantiate these observations. However, in a 19year old male patient there was a dramatic and unequivocal improvement. This patient had a long history of difficult, and at times violent, behaviour. During treatment with betaine, he stopped drinking heavily, acquired a part-time job, and in the words of his mother "became pleasant to live with." This change persisted throughout the whole of the treatment period. 3 patients had a history of bronchial asthma, and 2 of them (age 32y, 13y) had had frequent attacks. Apart from I attack in the early post-treatment period in patient 5, there were no additional attacks of asthma in these 2 patients during treatment."		

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease				Hcy level	Treatmnt and biochem etc contexts
Dudman NPB, Wilcken DEL (1983); Clinica Chimica Acta; Prince Henry Hospital, Sydney, Australia.	14 CBS--: (by enzyme, Hcy-uria, high methionine), aged 4-53y, (6 VitB6-nonresponsives, 8 VitB6-(partially?) responsives), And, 44 Controls: Matched for age, sex and smoking	Ratios CBS--:Normal, +/-SD				No details	Ambiguous suggestion of no treatment (refer to a cbl--patient not presented here) , but considering their details study above, & dates, maybe treatment was as above?....
		Plasma p-Phenylene-diamine oxidase	Plasma Total Copper	Plasma Caeruloplasmin	Erythrocyte Superoxide Dismutase		
		1.38+/- 0.21	1.40+/-0.15	1.28+/-0.22	0.96+/-0.15		
		P<.001	P<.001	P<.001	nss		

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Boers GHJ et al (1983); Metabolism U Nijmegen Nijmegan, Netherlands	8 CBS--: (by enzyme, Hcy-uria, high methionine), aged 20-50y), (mostly non-, with some partially-at-best-, VitB6-responsiveness?..)	Age at diagnosis: 20-50y Ectopia Lentis: 6/8 Myopia: 5/8, 4 with Ect Lentis Glaucoma: 3/8, all with Ect Lentis Dolichostenomelia: 3/8, all with Ect Lentis Arachnodactyly: 4/8, all EL, 2 with Dol Osteoporosis: 7/8, 5 with Ect Lentis Scoliosis: 6/8 all with Ost, 5 EL Thromboembolism: 3/8, all E L, all EEG Thrombophlebitis: 2/8, all with Ect Lentis Abnormal Arterial Blood Flow: all, 3 severe Convulsions: 2/8, 1 Thro'emb, all Thro'phl EEG abnormalities: 4/8, Psych'ic Illness: 2/8, all EEG, 1 Thro'emb (Continues)	(see continuation below)	Skin fibroblast CBS activities: CBS-- before VitB6: .03-0.14 Normal before VitB6: 3.6-26 CBS-- after VitB6: 0.10-0.56 Normal after VitB6: 4.9-32 (mostly non-, with some partially-at-best-, VitB6-responsiveness?…)
(Continues)				

(Continued)	(Continued)	Methionine	Cysteine("fCys")	Cystine	Hcy-Cys	Homocystine		
Boers GHJ et al (1983); Metabolism U Nijmegen Nijmegan, Netherlands	8 CBS--: (by enzyme, Hcy-uria, high methionine), aged 20-50y),	33uM	112uM	52uM	8uM	0.5uM	fHcy… = 9uM, tHcy = 49uM	VitB6 750mg/d for >1year
	(mostly non-, with some partially-at-best-, VitB6-responsiveness?..)	76uM	96uM	29uM	38uM	29uM	fHcy… = 96uM, tHcy = 136uM	After ceasing (VitB6 750mg/d for >1year) for 4weeks
	20 Controls: Adult	28uM+/-2	114uM	56uM+/-2	2.2uM+/-0.4	ND	fHcy ND, tHcy approx 10uM?…	
		(Continues)						

(Continued) Boers GHJ et al (1983); Metabolism U Nijmegen Nijmegan, Netherlands	Further Results: Following an oral methionine-loading at 0.1mg/kgBW (more than 6* the daily requirement in one lot, making the absolute values of the results somewhat irrelevant),whilst **on** VitB6 treatment the distribution of the cysteine between cystine and Hcy-Cys was changed from 52uM:8uM to 19uM:41uM pretty much linearly over 8hours. The total ("free") cysteine "fCys" is therefore much the same, but it's distribution moves to Hcy-Cys as the Hcy level rises in response to the methionine load's (plasma methionine up from 33uM to roughly 1000-600uM decrease across the 8hours) increase of the fHcy from approx 9uM to approx 130uM increase across the 8hours . Following an oral methionine-loading at 0.2mg/kgBW (more than 12* the daily requirement in one lot, making the absolute values of the results fairly irrelevant),whilst **not on** VitB6 treatment the distribution of the cysteine between cystine and Hcy-Cys was changed from 29uM:38uM to 9.5uM:43 pretty much linearly over 8hours. The net ("free") cysteine "fCys" is therefore much the same, but it's distribution moves to Hcy-Cys as the Hcy level rises in response to the methionine load's (plasma methionine up from 76uM to roughly 1000-750uM decrease across the 8hours) increase of the fHcy from approx 96uM to approx 180uM increase across the 8hours. So the qualitative effect of adding methionine, or removing the VitB6 therapy are similar, as expected, as the increased Hcy takes up more cysteine. The absolute methionine values will need to be compared with those attending ie methionine- unrestricted diets in order to make propose inferences regarding cysteine….

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Jackson GM et al (1984); Am Heart J; U California, San Diego, USA	1 CBS-- case	Previously recurrent thromboembolic events. At age 45y, Hcy-uria diagnosed, skin fibroblast enzyme assay No thromboembolic events in 5years prior to admission for following op "A 56y-old man was admitted for resection of an asymptomatic squamous cell carcinoma lung tumour ….All preoperative laboratory tests were normal including coagulation studies (prothrombin time 11.3sec/ control 11.3sec; partial thromboplastin time 32.5sec/ control 29.6sec; platelet count 310,000/mm^3…… Right upper lobectomy was performed under halothane anesthesia. Pyridoxine supplement was withheld. Postoperative complications included new onset atrial fibrillation, adult respiratory distress syndrome, and unexplained fever…..By the third postoperative day he was unresponsive to verbal command…..Three days later bleeding was noted with concomitant platelet count of 11,000/mm^3, prothrombin time 15.7sec/ control 11sec; partial thromboplastin time 34.6sec/ control 29sec …..On the seventh postoperative day homocystinuria and methioninuria. Pyridoxine Supplementation was resumed and folic acid and dipyridamole were provided. Neurologic examination on the tenth postoperative day was consistent with pontine, right frontal cortical, and left frontoparietal cortical ischemia. The following day he had the second episode of atrial fibrillation. By the thirteenth postoperative day platelet count rose to 158,000/mm^3, and the patient was again awake, alert, and responding to verbal command. The next day he was unresponsive to pain, quadriplegic, with central neurogenic hyperventilation, bilateral papilledema, and asymmetric unreactive pupils. Apneusis was followed by ataxic respirations and death. Postmortem….Several verrucous, fibrin vegetations were present on the tricuspid, pulmonic, and aortic valves. These were consistent with noninfective thrombotic endocarditis. An old myocardial infarct and one about two weeks old were present in the lateral wall of the left ventricle. Several pale cortical infarcts were seen within each kidney. A focal infarct was seen in the spleen. The brain weighed 1670g. The cerebral cortical surfaces were edematous, soft and showed scattered subpial hemorrhages. The sectioned cerebral hemispheres showed confluent petechial hemorrhages limited to the cortical grey matter. Microscopic examination showed many hemorrhagic and nonhemorrhagic cortical infarcts varying in age from hours to days. Rarely, thrombi were present within the lumen of small parenchymal vessels. No lesions were seen in the brainstem or cerebellum….This report is the first to demonstrate disseminated postoperative thromboembolism with thrombocytopenia and microangiopathic features in a patient with VitB6-responsive CBS-- in whom VitB6 supplement was withheld….measures to prevent intraoperative hemostasis, and perioperative provision of antiplatelet therapy, dietary and/or vitamin therapy, and adequate hydration seem prudent."	"Plasma Hcy(ine) normal'd" ? ? ? ? ? ? ? ? ? Hcy-uria present, Equates to tHcy > 40uM ? ? ? ? ? ?	VitB6 "pharma-cologic doses" VitB6 witheld VitB6 "pharma-cologic doses" resumed

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Newman G Mitchell JRA (1984); Quart J Med; U Hospital, Nottingham UK	2 CBS--siblings	A 20y old woman presented 2months after the delivery of her first child. Labour had been induced after a small ante-partum Hemorrhage but the pregnancy was otherwise normal. The vaginal delivery was complicated by a retained placenta which was removed manually under general anaesthesia. She remained well until 1month post-partum when she noticed that both her feet felt cold and numb. When walking she developed aching pain in both calves which abated after rest; during the next month her right leg gradually improved but she continued to have pain in the left. 2months post-partum she suddenly developed pain, numbness and paraesthesia in her left arm…. For 10minutes she also had intense vertigo and a disturbance of vision which she described as "like staring at a bright light". There was no relevant past medical history and she had never taken the contraceptive pill. During pregnancy she had taken ferrous sulphate and during labour she received oxytocin (Syntocinon), pethidine, promethazine and ergotamine with oxytocin (Syntometrine). After manual removal of the retained placenta a single dose of ergometrine was given intravenously. No drugs were given to suppress lactation and on admission she was taking inositol nicotinate (Hexopal)…. Initial investigations to identify a source of emboli, a vasculitis or a thrombotic tendency were negative. These included a chest radiograph, echocardiogram, blood cultures, full blood count and film, ESR, anti-thrombinIII level, fibrinolytic screen, VDRL, TPHA, and a search for immune complexes and cryoglobulins. An arch aortogram via the femoral route showed complete obstruction at the origins of the left subclavian and vertebral arteries and injection of contrast into the lower aorta showed marked narrowing of both external iliac arteries and occlusion of the right internal iliac artery. The left subclavian artery was explored; it was found to have a normal external appearance but to be occluded by a large thrombus which was removed. Histology showed this to be laminated platelet-leucocyte-fibrin material which was sterile on culture. The brachial pulse was restored but was lost again several hours after the operation. In spite of re-exploration, the removal of further thrombus and anticoagulation with heparin, the artery occluded yet again. She was subsequently anticoagulated with warfarin and made good progress apart from developing a left ulnar nerve palsy and a tender swollen left calf which had the clinical appearance of a typical deep vein thrombosis….. positive cyanide-nitroprusside reaction…confirmed aa analysis, but detailed clinical examination after the acute episode showed no stigmata of homocystinuria save for quivering of the iris of both eyes (iridodonesis) on slit-lamp examination. Her first-degree relatives were screened….1 of her brothers (CBS--). He was 6ft 2inches tall, with long thin fingers and face…apart from mild pectus excavatum and the Marfanoid features, physical examination was normal and there was no ocular abnormality….. Platelet behaviour was measured by our standard techniques. The tests were done on the patient 14days after her last operation, when she was on warfarin, but before pyridoxine treatment had been started. Her whole- blood platelet count was found to be very high 674,000/ul (normal range up to 450,000/ul). Her whole-blood platelet aggregation, as judged by the disappearance of single platelets from stirred whole blood to which EDTA had been added as anticoagulant was 20% in 6minutes (upper limit of normal 5%) but her ability to generate malondialdehyde (MDA) in clotted whole blood was normal at 12.5uM. Tests of platelet activity in separated platelet rich plasma were within our normal range (arachidonic acid-induced MDA production – 9,7nmol/10^9platelets; platelet release reaction, as judged by the 5HT liberated in response to challenge with ADP, adrenaline and collagen was normal). (Continues)		

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
(Continued) Newman G Mitchell JRA (1984); Quart J Med; U Hospital, Nottingham UK	2 CBS-- Sibl's	(Continued) Vessel biopsies were obtained from the patient while she was on warfarin and from her brother before he was on any medication. Segments of forearm vein were excised under local anesthesia and were immediately fixed both intact and after opening up. The blocks also included some small arteries. Scrutiny of the vessels by light, transmission, and where appropriate, scanning electron microscopy, showed them to be normal. In particular, normal endothelial cells were seen to be covering the intima with no breaks or defects….. Pyridoxine 250mg/day was added to her warfarin 1month after the episode of subclavian/vertebral occlusion.		
		Plasma: Methionine "Cystine"	Hcy(ine)	
	Sister	80uM Nil	20-100uM = >80-240 uM tHcy	Nil
		40uM 40uM	Nil = <40uM tHcy	VitB6 250mg/d
	Brother	10-100uM 10uM	40uM = >120uM tHcy	Nil
		40uM 60uM	Nil = <40uM tHcy	VitB6 250mg/d
		Her ulnar nerve palsy progressively improved, but when she became more active after leaving hospital, she experienced left calf claudication at 50yards. 2months later she could walk for 2miles on the flat without pain, although she still had some discomfort on hurrying up steep hills. This was matched by a return of the left dorsalis pedis pulse and a stronger left femoral pulse but there has been no return of the pulses in the left arm. She continues on pyridoxine 250mg daily and on warfarin to produce Thrombotest levels of 5-10%. We can document the contribution which the former has made to render the amino acid metabolism normal (overgeneralisation. DV) so we can confidently advise her to continue it indefinitely. What is less clear, however, is whether the correction of the homocystinemia with pyridoxine has now controlled her "thrombotic tendency" so that we could stop the anticoagulants. (Note: Consider the possibility that rebound hyposulfatism resulted from the cessation of the ferrous sulfate in the context of apparently no cysteine supplementation and the only minor activity of the other (methionine-metabolising) pathway generating endogenous sulfate – could this lead to heparin hyposulfation and a resultant increase in platelet stickiness?....DV) We are similarly uncertain as to the part played by pregnancy in her thrombotic episodes and do not know how to advise her about the risks of a second pregnancy, with or without anticoagulant cover. The brother has remained symptom free but has elected to start pyridoxine treatment. We are reporting these cases because we believe that the true prevalence of homcytinuria may have been underestimated by a tendency to seek it only in patients with typical features." Note: Their Summary and Discussion comments ("normal platelet behaviour", "no abnormalities of platelet function") seem most peculiar given the reported whole-blood platelet aggretation…..		

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Wilcken DEL et al (1985); Metabolism ; Prince Henry Hospital, Sydney, Australia.	6 CBS--: (by enzyme, Hcy-uria, high methionine), aged 11-53y, VitB6-y responsives).	Methionine+/-SD	Cysteine ("fCys"?)+/-SD	"fHcy"?…+/-SD.	VitB6 200mg/d Folate 5mg/day, following oral methionine load of 4g/m^2BodyArea, at:
		34uM+/-10	94uM+/-12	8.7uM+/-5.2 =49uM tHcy?..	0hours
		660uM+/-96	86uM+/-22	47uM+/-20 = 87uM tHcy?....	4hours
		560uM+/-104	92M+/-20	51uM+/-22 = 91uM tHcy?...	8hours
		500uM+/-105	88uM+/-19	48uM+/-23 = 88uM tHcy?....	12hours
		350uM+/-165	90uM+/-21	61uM+/-30 = 101uM tHcy?.	24hours
					(As above, but with Betaine2*3g/day in orange juice) *? 2-3weeks later: following oral methionine load of 4g/m^2BodyArea, at:
		107uM+/-156?15.6?	96uM+/-17	6.4uM+/-3.4 = 46uM tHcy?....	0hours
		760uM+/-130	101uM+/-19	17uM+/-7 = 57uM tHcy?...	4hours
		670uM+/-110	105uM+/-15	23uM+/-13 = 63uM tHcy?....	8hours
		580uM+/-120	103uM+/-23	23uM+/-17 = 63uM tHcy?...	12hours
		410uM+/-170	104uM+/-23	21uM+/-15 = 61uM tHcy?....	24hours
	17 Controls: Normal, aged 21-58y.				Following oral methionine load of 4g/m^2BodyArea, at:
		26uM+/-4	123uM+/-21	3.4uM+/-0.8 = <40uM tHcy?...	0hours
		560uM+/-70	134uM+/-22	14uM+/-6 = 54uM tHcy?....	4hours
		380uM+/-110	126uM+/-18	14uM+/-6 = 54uM tHcy?...	8hours
		230uM+/-120	125uM+/-16	11uM+/-4 = 51uM tHcy?....	12hours
		46uM+/-17	119uM+/-23	5.2uM+/-1 = 45uM tHcy?...	24hours
		Additional Results: Plasma Serine: CBS-- Pre-betaine: 94uM+/-19 CBS-- During-betaine: 117uM+/-14 p<.03 CBS-- During-betaine+MetLoad: average 34% increase over Pre-betaine+MetLoad			

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Authors, Journal, Site	Study Group	Disease
Mudd SH et al (1985); Am J Hum Genet; Worldwide survey coordinated from USA	"Data Base A standardized questionnaire was designed and mailed to each clinician known from a previous study (cites Mudd et al 1981) to be caring for patients with CBS--. Physicians were asked to complete a questionnaire for each such individual about whom they had appropriate information. To encourage participation, the questionnaire was kept relatively simple (cites availability through National Auxiliary Publications Service). …Further questions focused upon the factor(s) that led to ascertainment, whether the patient was responsive to VitB6, and upon the presence and age of appearance of major clinical manifestations. A detailed history of therapy was requested, as well as a reproductive history….Additional sources of information were identified by a review of the literature and by contacting centers around the world specializing in diagnosis and management of inborn errors of metabolism. Further, physician cooperation was solicited by notices in appropriate journals…some patients upon whom recent information could not be obtained….published material….only…details available to prove that they did not overlap. Data collection occurred during 1982 and early 1983…search for duplication…redundant information was deleted… For the present survey, updated information was received concerning 532 homocystinuric patients with proven or presumed CBS--. To this group was added material from an additional 97 patients obtained primarily from published reports (cites 37 references), bringing the total to 629 patients. All patients admitted to the study had been demonstrated to be excreting homocystine…either…enzyme assay or ….hypermethioninemia or dislocated optic lenses: Enzyme Assay: Yes No Ectopia Lentis + hyperMet 147(68%) 274(66%) Ectopia Lentis only 21(10%) 75(18%) Hyermethioninemia only 39(18%) 64(16%) Neither of the above 9(4%) 0(0%) Total 216(100%) 413(100%) 58 were discovered during screening of newborns, and an additional 88 were discovered by screening all siblings (the balance ascertained on clinical features)…. Of the 629 patients, 307 were females and 321 males…. Of the 629 patients, 231 (37%) were classified as biochemically responsive to VitB6 when not folate depleted; 231 (37%) were classified as nonresponsive to VitB6; 67 (11%) were judged intermediate in response; and 100 (16%) had not been classified. For subsequent analyses in this presentation (only the 231 VitB6-responsives and the 231 VitB6-nonresponsives were analysed)" Note: Newborn screening ascertainees 78% VitB6-nonresponsive; Untreated nonhypermethioninemic cases 90% VitB6-responsive	"Clinical Features Leading to Investigation for Homocystinuria (n = 472 not ascertained by newborn screening or proband sib screening, irrespective of VitB6-responsiveness): Clin Feature SoleCause ContribCause Total Ectopia Lentis 21% 65% 86% Mental Retard 4.0 52 56 Devel Retard 1.5 21 22.5 Early Thromboemb 1.1 15 16 Marfanoid Charact 0.9 36 37 Bony Abnormality 0.2 23 23 Seizures 0.2 3 3 Behav/Psychiatr 0 2.8 3 Other 0.4 10.6 11 " "Mental Capabilities …To eliminate the effect of very early therapy, data on patients discovered by newborn screening were not used in constructing these plots of IQ or in the alternative analyses of mental capabilities… (Note: The VitB6-responsives were still probably less-treated, and the VitB6-nonresponsives more-treated. dv) IQ Percentiles: 20%ile 40 60 80 VitB6-Responsive 60 72 82 94 VitB6-Nonrespons 42 51 60 68 Doc's Estim: GrossRetard MildRet LearnDis Av'g VitB6-Respon 5% 33% 11% 51% VitB6-Nonresp 38% 47% 4% 11% ….The effect of early treatment is illustrated in figure 2. IQs for VitB6-nonresponsive patients identified as neonates and treated from very early ages with methionine restriction, usually accompanied by L-cystine supplementation (early-treated), are compared to IQs of VItB6-nonresponsive patients not detected by newborn screening (late detected). If treated at all, very few of the latter patients had commenced therapy at ages of less than 1-2years" In that figure, whether having age at ascertainment or age at last follow-up on the x-axis, the IQs of the low-Met-CysSuppl-early-treated VitB6-nonresponsives lay about my visually estimated line of best fit from IQ 100 to IQ 80 left to right, while the non-early-treated VitB6-nonresponsives lay about their line of best fit from IQ 60 to IQ 57 left to right. There was minimal overlap, p<.001. (Continues)

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

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(Continued) (1) Mudd SH et al (1985); Am J Hum Genet; Worldwide survey coordinated from USA	As above.	(Continued 1) "Dislocation of Optic Lenses Data on ages of lens dislocation were analysed according to the Kaplan and Meier method, with the important stipulation that for each patient the only interval considered was that prior to initiation of treatment specific to CBS--. Hence, a patient was removed from the group at risk for lens dislocation (censored) at the time that he or she started such treatment…These plots demonstrate that for all groups of patients, there was a lag period of approximately 2years before appreciable lens dislocation occurred. After age 2, lens dislocation began to be detected but at different rates in VitB6-nonresponders and VitB6-responders, so that 50% of untreated VitB6-nonresponsive had dislocated lenses by age 6y, whereas for untreated BitB6-responsive patients, 50% dislocation was attained at approximately age 10y." n — Lens Dislocation: Expected — Observed — P VitB6-responsive Late-Detected treated only and continuously by VitB6+/-Folate — 24 — 8.4 — 5 — nss VitB6-nonresp'v Late-Detected treated only and continuously by Methionine restriction +/- Cysteine supplementation — 10 — 3.6 — 4 — nss VitB6-nonresp'v Early- Detected treated only and continuously by Methionine restriction +/- Cysteine supplementation — 30 — 11.7 — 3 — <.001 VitB6-nonresp'v Late-Detected treated only and continuously by Methionine restriction +/- Cysteine supplementation +/- (VitB6 and/or Folate) — 13 — 4.6 — 7 — nss VitB6-nonresp'v Early- Detected treated only and continuously by Methionine restriction +/- Cysteine supplementation +/- (VitB6 and/or Folate) — 31 — 12.5 — 4 — <.001 VitB6-responsive Late-Detected treated varyingly in all regards by VitB6+/-Folate — 26 — 10.8 — 5 — <.05 VitB6-nonresp'v Late-Detected treated varyingly in all regards by Methionine restriction +/- Cysteine supplementation +/- (VitB6 and/or Folate) — 23 — 6.9 — 9 — nss VitB6-nonresp'v Early- Detected treated varyingly in all regards by Methionine restriction +/- Cysteine supplementation +/- (VitB6 and/or Folate) — 30 — 16.2 — 6 — <.001

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(Continued) (2) Mudd SH et al (1985); Am J Hum Genet; Worldwide survey coordinated from USA	As above.	(Continued 2) "Thromboembolic Events From the thromboembolic events originally reported, 9 described as "possible" or "partial" and 2 that were only questionably related to the underlying condition were deleted. There remained a total of 253 events, occurring in 158 patients. No reported events occurred in 471 patients. Of these 253 events, 81 (32%) were cerebrovascular events, 130 (51%) affected peripheral veins (with 32 resulting in pulmonary embolism), 10 (4%) produced myocardial infarctions, 28(11%) affected peripheral arteries, and 4 fell into none of these categories. The distributions of these types of thromboembolic events were only marginally related to the VitB6-responsivity of the patients….. (versus Ectopia Lentis. dv) The lag for thromboembolism is longer: 8-12years before maximal rates are attained. The rates of occurrence of thromboembolic events are far less. For example, for the total group, the chances of suffering such an event were only about 25% by age 16y and 50% by age 29y. While the overall curves for VitB6-responders and for VitB6-nonresponders remain significantly different from each other (P = .02) the extent of the difference is not as striking as the case of lens dislocation….. Sufficient data were not available to permit construction of time-to-event curves for untreated patients after first episodes. To see if vitamin or dietary therapy had any major effect on the rate of occurrence of thromboembolic events subsequent to the first, it was therefore necessary to use a simplified analysis that included the assumption that the rates of such events is constant regardless of the age of the patient…..VitB6-responders off relevant treatment had 24 events during 3,744months of exposure and 7 events during 2028months of exposure on pyridoxine treatment (with or without folate), yielding rates of .08 and .04 events per year, respectively. VitB6-nonresponders off relevant treatment had 11 events during 1,264months of exposure and 4 events during 836months on methionine restriction (more often with cysteine than without. dv). The corresponding rates were 0.10 and .06 events per year. n Thromboembolism: Expected Observed P VitB6-responsive Late-Detected treated only and continuously by VitB6+/-Folate 120 14.9 3 <.001 VitB6-nonresp'v Late-Detected treated only and continuously by Methionine restriction +/- Cysteine supplementation 26 2.1 1 nss VitB6-nonresp'v Early- Detected treated only and continuously by Methionine restriction +/- Cysteine supplementation 30 1.7 0 nss VitB6-nonresp'v Late-Detected treated only and continuously by Methionine restriction +/- Cysteine supplementation +/- (VitB6 and/or Folate) 41 4.1 2 nss VitB6-nonresp'v Early- Detected treated only and continuously by Methionine restriction +/- Cysteine supplementation +/- (VitB6 and/or Folate) 32 2.1 0 nss VitB6-responsive Late-Detected treated varyingly in all regards by VitB6+/-Folate 135 19.9 4 <.001 VitB6-nonresp'v Late-Detected treated varyingly in all regards by Methionine restriction +/- Cysteine supplementation +/- (VitB6 and/or Folate) 68 6.2 8 nss VitB6-nonresp'v Early- Detected treated varyingly in all regards by Methionine restriction +/- Cysteine supplementation +/- (VitB6 and/or Folate) 41 2.9 1 nss" "Because many of the early-treated VitB6-nonresponders have not yet attained the ages when thromboembolic events are most likely to occur, few events were expected among this group. Therefore, the fact that almost none have occurred, although encouraging, is not yet proof of the efficacy of treatment." (Continues)

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(Continued) (3) Mudd SH et al (1985); Am J Hum Genet; Worldwide survey coordinated from USA	As above.	(Continued 3) "Osteoporosis Osteoporosis on the basis of a lateral radiograph of the spine was the definition of this finding. Time-to-event graphs for such spinal osteoporosis again demonstrate a progressive appearance that is significantly less rapid in VitB6-responsive patients than in VitBb-nonresponsive patients (P<.002)." Seizures Among all patients in the late-detected group, 114 were reported to have had seizures, and 422 were said not to have had seizures (incidence 21.3%). Among late-detected VitB6-responsive patients the incidence was 36/214 (16.8%), and among late-detected VitB6-nonresponsive patients the incidence was 43/184 (23.4%). Although this difference is not statistically significant, time-to-event curves indicated that seizure onset tended to occur somewhat earlier in untreated VitB6-nonresponsive (20% seizures by age 12y) than in untreated VitB6-responsive patients (20% by age 21y) (P = .04). Mortality Of the 629 patients covered in this study, 64 were deceased. The cause of death was unknown for three patients, and two died for reasons apparently unrelated to CBS—(accidental drowning at age 8; metastatic abdominal cancer at age 61). Of the remaining 59 deaths, thromboembolism was known to be the chief causative factor in 42 (71%) and was a probable, but less clearly established, major contributing factor in at least 5 others (8%). Pneumonia, other pulmonary infections, or sepsis were reported as the cause of death in 7 patients (12%). The latter were all grossly mentally retarded with IQs less than 50. For 5 patients, the causes were of uncertain relationship to the underlying disorder (heart failure at 15y in a severely retarded patient; subarachnoid hemorrhage at age 10 from two aneurysms of the basilar artery; spongy degeneration of the central nervous system with toxic sepsis; pulmonary hemorrhage in a 36d old infant; suicide at age 18).....Mortality among patients classified with regard to VitB6-response was 5.5% (29 of 529 patients). Survival for VitB6-responders was significantly higher than that for VitB6-nonresponders (P<.0001). For example, the expected mortality at age 20 was less than 5% among those responsive to VitB6 and approximately 20% in those not responsive" Ascertainment Bias ...."(1) Mental Capabilities. The "complete-sibship-screened" group of VitB6-responsive patients had a mean IQ 21 pints higher than the VitB6-responsive probands, a difference which was statistically significant. When IQ scores were not available, however, the proportions of each of these groups judged to be mentally retarded were not significantly different. Among VitB6-nonresponsive patients, no significant difference was present in the mean IQ scores for the "complete-sibship-screened" group compared with the probands, but only 65% of the former were judged to be retarded, as compared to 91% of the probands (P<.05) (2) Seizures. In the "complete-sibship-screened" group, seizures were reported in 16 of 85 patients (19%), an incidence not significantly different from that in the proband group: 98 of 449 (22%). (3) Lens dislocation. There were no statistically significant differences in the time-to-event curves for any of the "complete-sibship-screened" groups when compared with the proband groups. (4) First thromboembolic events. The results were the same as those for lenses. (5) Osteoporosis. The results were the same as for lenses and first thromboembolic events, except that for VitB6-responders the time of detection of osteoporosis in the "complete-sibship-screened" group was slightly delayed compared with that in the proband group (60% undetected vs 45% undetected respectively) (P = .04)..... .... "Data from screening programs of newborns furnish one means to arrive at a crude estimate of the possible magnitude of usch underascertainment. This was done for the USA, the country with the greatest number of patients reported (37% of all those classified as either VitB6-non/responsive). Combination of the results from screening programs of newborns in the Rocky Mountain states, New England, and Maryland and Delaware (various citations) leads to a rate of detection of CBS-- of 1/209,000 newborns (total 3+ million screened). Extrapolating this combined ratio to the country as a whole, assuming from the results reported here that 78% of those detected are VitB6-nonresponsive and taking into account the age structure of the population of the USA and the less than normal survival of VitB6-nonresponsive, one may estimate that in the USA the number of living VitB6-nonresponsive CBS-- persons <=25y of age should be 299, and incidence of 1/738,000 persons in the total population. Reports were obtained for the survey concerning 63 patients of this sort, or 1/3,500,000. Although the above estimate is no better than a very rough approximation, it appears that a substantial proportion of potential VitB6-nonresponsive subjects in the USA have been both diagnosed and reported on in the present survey. However, probably an even greater proportion remain to be diagnosed or (in our opinion less likely) are known to physicians but were not reported upon. .....(Continues)." (Continued)

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(Continued) (4) Mudd SH et al (1985); Am J Hum Genet; Worldwide survey coordinated from USA	As above.	(Continued 4) (Continued) Ascertainment Bias ….."The analogous calculations for VitB6-responders indicates that in the USA there should be only 55 such patients alive at <=25y of age (1/4,700,00). The low estimate is due to the fact that only 13% of patients detected in screening of newborns have been VitB6-responsive. In actuality, reports were obtained upon 47 such patients… (a little faulty reasoning. DV)…., these combined results support the likelihood that VitB6-responders are being missed in current screening programs of newborns, so that the above estimate of the potential number of these patients is probably too low (conclusion nonetheless correct. DV)." "Among the patients in this survey who had been detected by screening of newborns, and classified as to VitB6-responsiveness, only 13% were VitB6-responsive. Among late-detected patients, the corresponding value was 47%." "The USA provides a useful example for this sort of calculation, not anly because far more patients were reported from this country than from any other, but also because, in terms of patients reported per unit population, the USA fell at neither the upper nor the lower extreme of the range." Note: Mudd et al here have been fairly painstaking to address the issue of ascertainment bias, and readers interested in viewing a prologue to their work here may like to read their earlier work (cites Mudd et al 1981) and some of the discussion that followed on it in this regard (Swift and Morrell 1983 (), and Mudd et al response). It may be safely assumed that this work here effectively supercedes their earlier work in every aspect, and so I do not deal with that work.

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Sartorio R et al (1986); J Inherit Metab Dis; U Naples, Naples, Italy.	8 CBS--: (by enzyme, Hcy-uria, aged ?), 7 VitB6-responsive 1 VitB6-nonresponsive 15 Controls, normal, adults	VitB6-responsives Protein-bound Hcy "fHcy" DAM 4.0uM ND BF 10.3uM ND DA 13.4uM ND SG 23.8uM traces MA 32.0uM ND SS 40.0uM traces SR 43.4uM traces VitB6-nonresponsive TG 60.0uM 61.9uM Normal Controls 4.3+/-1.5uM Where ND = None Detected, "fHcy" apparently also includes Hcy-Cys "For determination of protein-bound homocyst(e)ine in plasma, the method proposed by Kang and colleagues (1979) was modified as follows. Fasting plasma samples, obtained from heparinized venous blood by immediate centrifugation were mixed with sulphosalicylic acid to a final concentration of 4.54%. After centrifugation (3000g for 10min) protein precipitates from plasma were washed seven times in water by successive centrifugations. Washed precipitates were suspended in water to the original plasma volume and then mixed with hydroxide to a final concentration of 0.4%, to solubilize proteins. The solution was brought to pH 7 by adding 7ul of concentrated HCl, mixed with 2-ME (1/10 v/v) and incubated at 37C for 120min. Proteins were reprecipitated with sulphosalicylic acid as indicated above and the clear supernatants were used for amino acid analysis. Amino acid analysis was carried out on a Becknman Model 119 CL Amino Acid Analyzer, according to the following conditions: 1st buffer: 0.2M lithium citrate, pH 2.83; 2nd buffer: 0.2M lithium citrate, pH 3.70; buffer change: 50min; column temperature: 40 and 65C; temperature change: 43min."	(derived) 4-10uM? 11-20uM? 14-20uM? 24-40uM? 32-40uM? 45-55uM? 50-60uM? <180uM 4-10uM?	 VitB6 " " " " " " Betaine
Abbott et al (1987); Am J Med Gen; Johns Hopkins U, Baltimore, Marylands, USA.	63 CBS--	"Our sample consisted of 63 patients with homocystinuria. All had typical clinical manifestations in at least three of four systems (ocular, skeletal, vascular, and central nervous system) and either a positive cyanide-nitroprusside test of urine, elevated plasma homocysteine, and methionine, or deficient CBS activity in cultured skin fibroblasts. In no patient was psychiatric aberration a criterion for diagnosis….36 males 27 females 45 sibships, mean age at diagnosis 19y…..mean age at psychiatric assessment 20y range 10-31y." PsychDis IQ<80 IQ>=80 VitB6-resp VitB6-nonresp B6?resp MajDepr 3% 15% 7% 7% 14% BehDisord 33% 3% 7% 33% 19% ObsComp 10% 0% 4% 13% 0% Personality 20% 18% 22% 20% 14% TotalPsy 66% 36% 40% 73% 47% Total % 100% 100% 100% 100% 100% Cases n 30 33 27 15 21	Not Given	Not given, VitB6 for the VitB6-resp, LowMet +/- Cys for the VitB6-nonresp? Folate? VitB12? From unknown age….

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Kempster et al (1988) J Neurol Neurosurg Psychiatry; University College London, UK	3* CBS--	"Case 1: An 11y old boy with intellectual impairment (IQ = 60), Marfanoid skeletal deformity, osteoporosis and bilateral lens dislocation was found to have homocystinuria. The biochemical changes were not improved by treatment with pyridoxine. At the age of 18y he developed spasmodic torticollis followed by truncal and upper limb dystonia. He had prominent retrocollis and truncal hyperextension which were exacerbated by walking. Tetrabenzine and anticholinergic agents had no effect on the dydtonia although bromocriptine produced a modest improvement. He died at the age of 22 from bronchopneumonia secondary to severe neurological disability. Neuropathological examination. The brain weighed 1520g and showed no macroscopic abnormalities on external and cut surfaces. The vessels of the circle of Willis were of normal configuration and serial sections of the main cerebral vessels revealed no sign of organised thrombus. Histological examination with haematoxylin and eosin staining showed no evidence of thrombosis affecting small intraparenchymal vessels. Sections through areas of frontal lobe convexity and orbital cerebral cortex and temporal lobe cerebral cortex were normal with respect to the thickness of the cortical ribbon, and cell numbers, type and layering; there was no sign of gliosis. Sections of caudate nucleus, putamen, globus pallidus, subthalamic nucleus, red nucleus and substantia nigra were normally pigmented. Cell populations in all of these areas were assessed semi-quantitatively in luxol fast blue/cresyl violet sections; standard areas were examined using eyepiece graticule and stage micrometer to check magnification and compared to an age and sex matched control. This did not indicate any significant loss of neurons. Case 2: This girl presented at age 7years with mental retardation (IQ = 60) and bilateral lens dislocation. At that time a diagnosis of pyridoxine-resistant homocystinuria was made. At age 9, upper right limb tremor and hypertonia associated with abnormal posture of the right shoulder were observed and over the subsequent years she developed progressive dystonia affecting limbs, neck, trunk and tongue associated with dysarthria, generalised hypertonia, hyperreflexia and a shuffling gait. A cranial computed tomographic scan at the age of 20 was normal apart from a small cortical area of low density in the right temporal region. Now aged 26, she has persistent generalised dystonia which has proved resistant to treatment with haloperidol, Lioresal, bromocriptine and clonazepam. Case 3: This boy presented at the age of 4years with marked developmental delay and bilateral lens dislocation. Pyridoxine resistant homocystinuria was diagnosed. At age 9years he began to have tonic-clonic and akinetic seizures, which were treated with phenytoin and sodium valproate. He developed a Marfanoid body habitus and was severely intellectually impaired (IQ<50). At age 5years he first showed a tendency to walk on his toes and by the age of 10 his gait was unsteady, he was dysarthric and there was generalised hyperreflexia. Thereafter gradual evolution of involuntary movements affecting neck, trunk and lower limbs occurred. At the present time, aged 19, he has dystonic posturing of the neck and feet with superimposed rapid torsional neck movements and choreiform lower limb movements. A cranial CT scan at the age of 13 was normal: electroencephalography showed bilaterally synchronous paroxysms of polyspike and wave activity. Haloperidol and clonazepam were ineffective in controlling the disorder." "Discussion …..our findings suggest that dystonia in homocystinuria may relate to functional disturbance in neurotransmission rather than to detectable neuronal damage…….homocysteic acid, an oxidation product of Hcy is known to have excitotoxic activity in vitro (cites Olney 1974)…shows a similar glutamate displacing pattern to N-methyl-D-aspartate in neural tissue……Taurine has an inhibitory action in the central nervous system (cites Kuriyama et al 1978) and may modulate neurotransmitter release, including that of dopamine (cites Okuyama S et al 1985). Reduction in basal ganglia taurine is therefore a possible cause for dystonia in homocystinuria."

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Wiley C et al (1988); Metabolism; Prince Henry Hospital, Sydney, New South Wales, Australia.		fCys boundCys boundCys tCys fHcy boundHcy boundHcy	tHcy	
	6 VitB6-responsiv CBS--	96uM 1.7umol/g 126uM >222uM 6.4uM 0.24umol/g 18uM	>24uM	VitB6 + folate + betaine
	6 VitB6-responsiv CBS--	94uM 1.4umol/g 104uM >200uM 11.1uM 0.31umol/g 23uM	>34uM	VitB6 + folate e
	3 VitB6-partlyresp CBS--	77uM 0.76umol/g 56uM >133uM 41uM 0.52umol/g 38uM	>79uM	VitB6 + folate
	1 VitB6-partlyresp CBS--	46uM 0.24umol/g 18uM >64uM 240uM 1.85umol/g 140uM	>380uM	Nil
	7 VitB6-nonresp CBS--	82uM 1.1umol/g 81uM >163uM 52uM 0.68umol/g 50uM	>102uM	VitB6 + folate + betaine
	6 VitB6-nonresp CBS--	56uM 0.23umol/g 17uM >73uM 175uM 1.1umol/g 81uM	>256uM	VitB6 + folate e
	21 Controls: fCys and fHcy data only available for 7 of these, but boundCys and boundHcy were respectively very similar for the 7, 14, so given here as one value(s).	114uM 2.3umol/g 170uM >284uM 3.0uM 0.15umol/g 11uM Note: The second "boundCys/Hcy" (uM) in each of the sections above was derived by me from multiplication of their first "boundCys/Hcy" (umol/g, where g is grams of protein) by the factor of 74 g protein per litre of Serum (midrange of Mosby's Manual of Diagnostic Laboratory Tests (Pagana and Pagana 2002 () 64-83g/L for normal adult). The tCys/Hcy was then derived by me by adding the uM units for bound and free Cys/Hcy – the derivations are therefore not quite correct from the volumetric aspect, but are close enough to give the useful comparisons visible between the groups. Note: Due to the degree of any methionine restriction/ lowness of protein in diets, and particularly the use of supplementary cystine, being probably as poorly documented here as generally elsewhere, and note any cystine supplementation being more facile of compliance than food modification/restriction, it may'nt be assumed that these are as given, nor that they are equivalent between any two of the six patient groups. "Samples of venous blood were drawn after an overnight fast or during the period of an oral methionine load. The plasma was immediately separated (700g, 10min, 4C) and deproteinized by addition of 20% sulfosalicylic acid to give a final concentration of 4%. The deproteinized plasma preparation was centrifuged (13,000g, 10min) and the supernatant stored at -16C for aa analysis…..The protein pellet was washed twice twice with distilled water, covered with distilled water and stored at -16C….Levels of bound Cys and bound Hcy were estimated using 15-20mg of dried protein by the method of Smolin et al (cites Smolin and Benevenga 1982)…The following changes were made to the published method: reduction occurred for 60min; 50uL of octanol was used; the reaction was run under humidified nitrogen and consequently no adjustment ot volume of solution was made; and particulate matter was removed from samples by centrifugation (13.000g, 10min) and filtration (Amicon Seraclear filters….standards… the recovery for cysteine was mean 82%, and for Hcy mean 87%.....All results have been adjusted for these recoveries" (Continues)	>14uM	Nil
(Continues)				

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
(Continued) Wiley C et al (1988); Metabolism ; Prince Henry Hospital, Sydney, New South Wales, Australia.	See Above	The Authors provide 2 scatterplots, one with plasma free Cys (fCys) on the x axis, the other with plasma free Hcy (fHcy) on the x axis, the y axes being "Ratio: Bound Cys/Free Cys (Hcy) ((umol/g protein x 1000)/umol/l)" – note that the factor 1000 should have been 74 as noted above, so for the following discussion I have corrected their y axes' s scales by dividing them by 1000/74 = 13.5. For the cysteine there is a slight curve ascending to the right, such that at plasma fCys 50uM the ratio = 0.3, at plasma fCys 75uM the ratio = 1.0, at plasma fCys 100uM the ratio is 1.4, at plasma fCys 125uM the ratio is 1.7, at plasma fCys 150uM the ratio is 1.9. For the homocysteine there is a quite sharp reverse-J-curve descending to the right, such that at plasma fHcy <10uM the ratio = >2.0 (max 6 at far left), at plasma fHcy 20uM the ratio = 1.3, at plasma fHcy 30uM the ratio is 1.1, at plasma fHcy 50uM the ratio is 1.0, at plasma fHcy 100uM the ratio is 0.9, at plasma fHcy 200uM the ratio is 0.6 It may be concluded that Hcy has a greater affinity than Cys for plasma proteins It may be concluded that my operating assumption that Hcy<40uM is bound to protein and thereafter free as Hcy(ine) or Hcy-Cys is simplistic (and that not surprisingly), as tHcy goes up to say 200uM, then boundHcy goes up to say 75uM, so that where I have added only 40uM to higher values of fHcy in the literature to derive a rough tHcy, it may be taken that the tHcy value is lower than it ought be, by as much as 100uM at say 400uM fHcy. At low ie trace or nil values of fHcy the assumption of tHcy<40uM is roughly correct enough for most useful purposes. Also the ramifications regarding the higher values ie in CBS-- are trivial for most useful purposes, and for mine in this work.
Wiley C et al (1989); Metabolism ; Prince Henry Hospital, Sydney, New South Wales, Australia.	See next column	6 CBS--VitB6-responsive patients on VitB6 + folate +/- betaine & 7 CBS--VitB6-non-responsive patients on VitB6 + folate +/- betaine from the study immediately above (**which** groups of patients thereof ???…unknown) The findings of this study add little to those of their study detailed immediately above, for my purposes here, other than that the same general trends notable in their study above with respect to the distribution of Hcy and Cys between free and protein-bound plasma pools, applies also 4hours after an oral methionine load of 4g/M^2 body surface area.
Brattstrom L et al (1989); J Inher Metab Dis; U Lund U Gothenburg , Sweden		Age FactorVIIAntigen AntithrombinIIIActivity Post-methionine-load tHcy Fasting tHcy CBS--1 30y 80% 81% 244uM CBS--2 18y 82% 105% 54uM CBS--3 38y 110% 125% 178uM 20 CBS-+ 56+/-12y 111+/-20% 119+/-9% 51+/-27uM*** 13+/-5uM* 46 normal CBS++ 52+/-7y 113+/-22% 120+/-10% 21+/-8uM 11+/-3uM 9 Downs Syndrome CBS+++ 41+/-4y 122+/-12% 102+/-2.3% *** 22+/-8uM 10+/-2uM * = P<.05; *** = P<.001 compared to normal CBS++ contrrols Methionine load was 3.84g/M^2 body surface area. CBS-- all had ectopia lentis, hypermethioninemia and Hcy-uria, Treated with methionine restriction, VitB6 and folate. Downs Syndrome by chromosome analysis . CBS-+ obligate heterozygote parents of a CBS--.

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Cochran FB et al (1990); Am J Med Gen ; U California, San Francisco, USA.		"The Propositus is the only child of non-cansanguinious parents. The diagnosis of asthma was made in infancy with a history of frequent exacerbations and hospitalizations since age 8y. He maintained an "A" average in school and participated in the school band, despite frequent absences for asthma treatment. At age 14y he was hospitalized because of recurrent left pneumothoraces requiring chest tubes. Soon thereafter he developed a right pneumothorax and staphylococcal pneumonia, and presented with headache, nausea, vomiting, syncope, diplopia, and dizziness. CT scan (head) showed diffuse white matter edema consistent with psuedotumour cerebri. He was transferred to the U of California SF because of increasing intracranial pressure and bradycardia. At the time of transfer he was being treated with theophylline, prednisone, Micronase, phenobarbiatal, erythromycin, vancomycin, Bronkosol, meperidine, hydroxyzine, and metaproterenol nebulizer…..Neurological examination was normal except for 2-3 beats of clonus bilaterally and right extensor plantar response (which later became flexor). There was a mild papilledema without hemorrhage or vessel abnormality. Visual acuity was 20/20 uncorrected, O.U. and visual fields were full with normal color vision. On lumbar puncture the opening pressure was 225mm H2O, and on closing pressure was 85mmH2O. Results of CSF studies were normal, and all CSF and blood cultures were sterile. Hypercoagulability workup showed no abnormality. Subsequently he developed empyemia, necrotizing pneumonia, deep venous thromboses, and hypertension treated with antibiotics, heparin, and anti-hypertensive medications, respectively. His symptoms of increased intracranial pressure responded to acetazolamide…. The diagnosis of homocystinuria was made on the basis of a urine cyanide nitroprusside reaction and elevated plasma homocystine and methionine …..CBS activity in cultured skin fibroblasts = 0.3% of control, and was not altered by the addition of pyridoxine. CT scan of the head showed decreased density of the white matter diffusely, with normal-appearing grey matter….small focus of low density…superior sagittal sinus….MRI confirms sagittal sinus thrombosis…. The delta sign, indicative of superior sagittal sinus thrombosis, was absent on repeat head CT scan 24days later. A methionine-restricted diet 10mg/kgBW/day was instituted, and the patient was begun on supplemental pyridoxine up to 500mg/day, and folate 0.5mg/day without change in plasma Hcy(ine). The addition of betaine 3g BID to the regimen resulted in a substantial reduction in plasma Hcy(ine) levels during the initial 4months of treatment	fHcy (assume) 42uM = tHcy >82uM fHcy (assume) 7uM = tHcy >47uM	Methioni 608uM Methioni 745uM See column 2 to left for therapies details
Rubba P et al (1990) ; Metabolism ; U Naples, Naples, Italy.	14 CBS-- 14 Contrls 13 CBS-+ 47 Contrls	Age AnkleSBP/ArmSBP<0.97 IliacArteryLesions CarotidArtLesions 20y 6legs (21%)* 5arteries (18%)* 0arteries (0%) 20y 0legs (0%) 0arteries (0%) 0arteries (0%) 46y 2legs(8%) 8arteries (31%)** 6arteries (23%)* 43y 3legs(3%) 2arteries (2%) 3arteries (3%) SBP = Systolic Blood Pressure * = P<.05; ** = P<.01 compared to normal CBS++ controls Controls clinically healthy U Naples admin staff volunteers, and some of their younger relatives, age-matched; CBS-+ obligate heterozygotes.	Not Given	All CBS-- on VitB6 600-900 mg/day, 1 VitB6-nonresp CBS--also on betaine 8g/day

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
Wagstaff J et al (1991); J Pediatrics; Harvard Medical School, Boston, Massachu-setts, USA	1 CBS--case	"The patient was born to a 28y old gravida 4, para 4 woman after a pregnancy complicated by emesis that was treated with pyridoxine. Both parents were of Portuguese-Azorean ancestry, and there was no known consanguinity. Results of neonatal screening for hypermethioninemia at age 14days were normal. The diet for the first 10weeks of life was commercial formula with iron. After an episode of gastroenteritis, the family changed the patient's diet to diluted cow's milk mixed with cornstarch and then boiled. After 1month of this diet, the patient became irritable with epistaxis and hematochezia developed. When seen at Children's Hospital at 4months of age, he was irritable and pale and had scattered petechiae……platelet count 12,000/mm3….bone marrow aspirates showed marked megaloblastosis….. The serum folate was 5.7nM (normal 7-36nM), the RBC folate was 20nM (normal 340-1360nM); serum VitB12 270pM (normal 150-660pM); Hcy(ine) 39uM (normally undetectable)…… The patients pancytopenia resolved with folic acid therapy. The subsequent addition of oral pyridoxine hydrochloride in doses as high as 500mg/day did not produce any substantial decrease in his plasma methionine or Hcy levels. He was subsequently fed a low-methionine diet supplemented with L-cystine. His methionine levels dropped to the normal range and homocyst(e)ine became undetectable. He has no evidence of lens dislocation, and his motor development at age 8months is within the normal range for age."
Monreal M et al (1991); J Cardiovasc Surg ; U Hospital Badalona, Bercelona, Spain.	1 CBS--case	"A 28y old woman presented at the Hospital with severe left limb ischemia. She had a 3year history of smoking 10 cigarettes a day and she did not take contraceptive pills. She was born from nonconsanguineous parents and when he was 8year old both lenses were removed because of ectopia lentis. Since 1985, she had experienced symptoms of intermittent claudication in her left leg, and two months before admission in April 1988, she was operated upon in another hospital, where a left femoroperoneal bypass was performed, but a few hours later this thrombosed. One month later the patient was referred to us. On admission, severe signs of ischemia were apparent in the left leg. Blood pressure was normal but the heart rate was persistently elevated (124bpm). No abnormalities were found on respiratory, abdominal and neurological examination. All the blood coagulation tests, blood cell counts and routine biochemical tests were also normal. Five days after admission we amputated three toes n the left foot. Nine days later, she developed sudden dyspnoea and chest pain. A ventilation-perfusion lung scan showed perfusion defects consistent with pulmonary embolism, and heparin therapy was started. Twelve days later, the heparin was switched to oral anticoagulants and at this time the patient developed pallor, cyanosis and rest pain in the left leg. Arteriography showed complete obstruction of the right popliteal artery…… Thus, tests for antinuclear, antitissue and antiphospholipid antibodies (including VDRL and anticardiolipin antibodies), as well as antithrombin III, protein-C and protein-S measurements were performed; all were either negative or within normal reference values. However….ectopia lentis….homocystinuria….. Subsequently, in September 1988, she underwent a supracondylar amputation of her left leg after many unsuccessful revascularization attempts. Sixteen month later, while being on VitB6 therapy, the patient remains well, and she has not, so far, presented any additional ischemic events."
Nordstrom M & Kjellstrom T (1992) ; Athero-sclerosis; U Lund, Malmo, Sweden.	7 CBS-- 14 CBS-+ 20 CBS++ Athero-sclerotics 29 CBS++ Contr Age>=45 20 CBS++ Contr Age<=44 7 CBS+++ Downs Syndrome	CBS activities (nmol/hour/mgProtein) (+/-SD) in skin punch biopsies Age / No added pyridoxal phosphate / With added pyridoxal phosphate 28+/-12y / 0.00 / .04 / Note: Unexamined for VitB6-response 52+/-9y / 0.88+/-0.7 / 2.31+/-2.0 / Note: Obligate heterozygote parents 52+/-9y / 1.5+/-1.2 / 2.90+/-2.4 / Note: Varied, no hepat, renal, diabet >=45y / 2.19+/-2.0 / 4.37+/-3.7 / Note: Clinically healthy screen volun <=44y / 7.79+/-3.7 / 17.8+/-10 / Note: Clinically healthy screen volun 42+/-17y / 3.35+/-1.7 / 7.34+/-4.1 Additional: Embryonic cells 14-16weeks gestation – 14.4+/-5.1, 25.4+/-9.6, respectively

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal,	Study Group	Disease	Hcy level	Treatment and biochem etc contexts
Mansoor MA et al (1993); Metabolism U Bergen, Bergen, Norway.	7 CBS--	Age/Sex CardiovascDis PlasmaMethionine	tHcy	
	Case 1	18y F Cerebrovascular 83uM	360uM	VitB6 160mg*3/day
	Case 2	20y F VenousThromboembol 158uM	360uM	VitB6 200mg*3/day
	Case 3	20y M VenousThromboembol 574uM	290uM	VitB6 450mg*3/day
	Case 4	3y F None 94uM	200uM	Folate 40mg*3/day
	Case 5	19y M Cerobrovascular 830uM	320uM	None
	Case 6	14y F None 320uM	240uM	None
	Case 7	17y F None 490uM	130uM	Folate 5mg*3/day + Betaine 12g/day
		Additional: The authors provide a graphing on log scale y axis, some salient overall features of which I deign to graciously here present: # Protein-bound Hcy is very roughly twice as much as each of reduced and oxidised Hcy such that if the latter two were combined to give fHcy, it would be very roughly equal to protein-bound Hcy. # Protein-bound Cys is very roughly equal to each of reduced and oxidised Hcy such that if the latter two were combined to give fCys, it would be very roughly twice protein-bound Cys. # Protein-bound "Cyst(e)inylglycine" is very roughly equal to each of reduced and oxidised "Cyst(e)inylglycine" such that if the latter two were combined to give f"Cyst(e)inylglycine", it would be very roughly twice protein-bound "Cyst(e)inylglycine". # tHcy very roughly thrice tCys, which is very roughly thrice # tHcy very roughly thrice tCys.		
Celermajer DS et al (1993) ; JACC ; Hospital for Sick Children, London, UK		Chol VesselSize FMD NTG RestFlow ReactHypem	tHcy	
	9 CBS-- children Age 11+/-1.3y 33%Male	4.1mM 4.0mm 2.8% 13% 219ml/min 349%	63uM (38-104)	"8 were taking a low-protein diet, 6 were taking VitB6, 7 were prescribed betaine, and all were taking folic acid supplements."
	18Control Children Age 12+/-0.7y 33%Male	4.2mM 4.2mm 9.0% 16% 204ml/min 391%	(7-19uM) ?....	"normal diet"
	P	0.74 0.45 <.0001 0.27 0.78 0.55		
		FMD = Flow Mediated Dilation NTG = Nitroglycerin-induced dilation Chol = Cholesterol Restflow = blood flow at rest ReactHypem = Reactive Hyperemia CBS—Detail: "In these children, the age at diagnosis was 4months to 7.5years (mean 4y) and the age at time of study was 4-17years (mean 11y). Of the 9 children, 7 (78%) had lens dislocation and 4 (44%) were at a special school because of mental retardation."		Note: "No child had symptoms or signs of vascular disease or ultrasound evidence of arterial narrowing or plaque formation in the vessel studied."

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Allen RH et al (1993); Metabolism U Colorado, Denver, Colorado, USA.	5 CBS--	Betaine NNDMGly NMGly Methionine Cystathionine tCys	tHcy	Betaine
	Case 1	24uM 2.9uM 2.3uM 22uM 74nM 300uM	19uM	No
	Case2	660uM 34uM 19uM 76uM 114nM 186uM	50uM	Yes
	Case 3	20uM 4.8uM 7.7uM 43uM 38nM 136uM	91uM	No
	Case 4	560uM 158uM 25uM 530uM 39nM 185uM	99uM	Yes
	Case 5	170uM 250uM 49uM 660uM 30uM 123uM	156uM	Yes
	Normal Range	17-73 2-6.6 0.6-2.7 13-43 65-300 200-360 NNDMGly = NNDimethylglycine, NMGly = NMethylglycine	5-16uM	
		"Serum samples from 60 subjects, 30 male, aged 18-65y, were obtained from healthy blood donors at the Belle Bonfils Blood Bank, Denver… .The diagnosis of CBS—was based on criteria that included increased serum levels of Hcy(ine), tHcy, and methionine, direct enzyme assays on cultured fibroblasts, and the presence of ectopic lenses." "Finally, in the course of our studies we have noted that many physicians are surprised to find that their patients' total homocysteine levels are still markedly increased even after their markedly increased serum homocystine levels (as measured with the amino acid analyser) have decreased to very low or undetectable levels following treatment with pyridoxine in the case of CBS-- …..The disparity between measurements of total homocysteine and those of homocystine is due to the fact that Hcy and Cys are monosulfides that compete with each other to form disulfides of Hcy-Hcy (homocystine), Cys-Cys (Cystine and cysteine bound to cysteine residues in protein), and Hcy-Cys (Hcy-Cys mixed disulfide and Hcy bound to cysteine residues in protein). Since the levels of Cys are relatively constant in CBS-- (Concentrations of total cysteine are actually often decreased in these patients and increase toward or to normal with standard therapy; this has the effect of magnifying the differences in fold-response of tHcy versus Hcy(ine)) levels of Hcy(ine) increase exponentially as tHcy increases and decrease exponentially as tHcy decreases with therapy. This relationship is illustrated in Table 4, where it can be seen that a decrease in tHcy of only 6fold or 20fold results in respective decreases of 21fold and 210fold in Hcy(ine) level…. Table 4 Calculated Values for Serum tHcy, Hcy(ine), Cystine, and Hcy-Cys in a Hypothetical Patient with a Constant tCys level of 300uM and tHcy levels of 300, 150, 50, 30, and 15uM tCys tHcy TotalDimer Hcy(ine) Cystine Hcy-Cys Ammt Decrease Ammt Decr Ammt Incr Ammt Decr 300 300 nil 300 75 nil 75 nil 150 nil 300 150 2fold 225 25 3fold 100 1.3fold 100 1.5fold 300 50 6fold 175 3.6 21fold 129 1.7fold 43 3.5fold 300 30 10fold 165 1.4 55fold 136 1.8fold 27 5.5fold 300 15 20fold 157.5 0.4 210fold 143 1.9fold 14 10fold Note. Results are based on the assumption that Cys and Hcy can only form disulfides with themselves and each other in a random manner. This example is an oversimplification since significant amounts of tCys and tHcy also form disulfides with Cys residues in albumin and other proteins and peptides. Nevertheless, it illustrates fully how Hcy(ine) changes exponentially as tHcy changes in a linear manner." Note: The great variation in not only the use of supplemental Cys in CBS-- treatment, but also the reporting/documentation thereof ought be kept in mind. A decrease in tCys to say 100uM would decrease the exponentiality of the Hcy(ine) changes but not abolish it.		

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal,	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Rubba P et al (1994) ; Stroke ; U Frederico II, Naples, Italy.		Wall Thicknesses of Carotid Arteries and Flow Velocities in Middle Cerebral Arteries and Heart Rate and Pulsatility Index AvMaxThick MaxThick AvSystolVel AvDiastolVel HR AvPulsatil		
	11 normal Controls Age 21-35	0.564mm 0.807mm 95cm/sec 46cm/sec 70bpm 0.80		
	12 CBS-- Age 8-42y	0.620mm 0.908mm 100cm/sec 48cm/sec 73bpm 0.82	tHcy <100uM?	CBS—received VitB6 since diagnosis for 3-5y – all but one were responsiv
	10 Familial Hyper-cholest'ia Age 4-49y	1.42mm 2.60mm 94cm/sec 37cm/sec 75bpm 1.02		
	P (Anova)	<.01 <.01 nss .01 nss <.01		
		"Quantitative B-mode ultrasound imaging and flow velocity evaluation with transcranial Doppler ultrasound"		
		"The diagnosis of CBS-- was based on the following criteria: (1) presence of Hcy in the urine and (2) abnormally low CBS activity in cultured skin fibroblasts and/or hypermethioninemia and hyperhomocystinemia with low or undetectable levels of cystinemia. Index cases were referred by local ophthalmologists after diagnosis of lens dislocation; the other affected patients were identified by screening the families of the index cases." (In all CBS--) "Ankle pressure estimated by the continuous-wave Doppler method was abnormally low and indicative of obliterating arterial disease of the lower limbs in 3 of 12. 4 of 12 had wall irregularities in the iliac arteries based on Duplex evaluation. None reported previous MInfarct, stroke, angina pectoris, intermitt claud."		
		"The mechanism underlying the thrombotic events that occur even in early-treated, pyridoxine-responsive patients is unknown. On the basis of our observations it is unlikely that typical atherosclerotic lesions precede thrombus formation in Hcy-uric patients within this age range. It is possible that the vascular lesions in more severe cases of Hcy-uria, in which patients have not been treated for several years, are to some extent different from those seen in our early-detected well-treated cases. However pathology studies in untreated patients who prematurely died of severe thromboembolic disease rarely show typical atherosclerotic lesions (Table 2). A more consistent finding in these patients is arterial dilatation, which has been suggested to precede the thrombotic event…		
Cite the following authors whose data they collate here:	CBS-- cases	Table 2. Gross pathology and histological findings in 12 Hcy-uric patients who prematurely died of cardiofvascular diseases Gross Pathology Histology Thrombi Arteries Veins Aneurysm IntimThick AbnElasFibr AbnMed Athero		
Gibson et al (1964)	1 case	Yes Yes Yes Yes Yes Yes Yes		
Carson et al (1965)	1 case	Yes Yes No Yes Yes Yes No		
Shimke et al (1965)	6 cases	Yes Yes Yes Yes Yes Yes No		
Carey et al (1968)	1 case	Yes No No Yes No Yes No		
McCully (1969)	1 case	Yes No No Yes Yes Yes No		
Hopkins et al (1969)	1 case	Yes Yes		
Almgren et al (1969)	1 case	Yes No Yes No Yes Yes Yes		

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Schienle HW et al (1995); Thromb Res; Philipps U Baldingerstr Marburg, Germany	2 CBS—cases	Case 1, male, for many years prior to age 23y ectopia lentis known, no Marfanoid signs, good intelligence occupation computer programmer, at age 23y knee op no complications, low prothrombin time (64%), low level of F VIIc (28%), aPTT normal, liver function tests normal, at age knee op + 5months stupor and seizures (12/89), superior sagittalis vein thrombosis diagnosed by MRI, AT III activity low, Heparin….aPTT prolonged to approx 90sec… discharged after 5weeks…dicoumarol not instituted because prothrombin time was prolonged (median quick value 53%). Antiepileptic therapy was given for 1year because of focal necrotic-ischemic changes in the occipital lobes… although heparin therapy was continued, thrombosis of calf and thigh veins with subsequent lung embolism occurred 2months later, CBS-- diagnosed VitB6 600mg/day, Folate 15mg/day started (response as tabulated below) heparin continued for another 1.5years patient does normal sports and studies computer science. Case 2, female, sister of case 1. ectopia lentis known for many years, no Marfanoid signs, following on the brothers experience , CBS-- diagnosed VitB6 600mg/day, Folate 15mg/day started. Plasma: fHcyPre fHcyPost MetPre MetPost Case 1 >280uM 0-40uM 75uM 30uM Case 2 >56uM 0uM 520uM 20-50uM "In patient 1 RiCoF (Von Willebrand Factor) was measured on 3 occasions during the clinical phase without thromboembolic disease and without vitamin treatment and found to be elevated to 224% of the normal value. During thromboembolic disease RiCoF was 336% of normal. During specific vitamin therapy the median value of RiCoF (n = 15) decreased to 144% of normal (66-168%). The time course of RiCoF shows that RiCoF dropped slowly to normal values. In patient 2, RiCoF was 168% on 2 occasions, whereas under vitamin therapy the median of 13 measurements was 108% of normal (66-168%)	tHcyPost 54uM 16uM	
Applegarth DA et al (1995); Eur J Pediatr; U British Columbia, Vancouver, Canada.	N = ? CBS--	"In many cases, the tHcy level rose to about 50uM befote any increase in plasma fHcy(ine) or Hcy-Cys could reliably be detected on the amino acid analyser system. In some patients, tHcy levels of about 20uM did demonstrate fHcy or Hcy-Cys, but in most patients the tHcy levels needed to be at least 50uM (about 4 times the upper limit of the control range) before any fHcy was detected."	fHcy = nil until tHcy >50uM	Low-methio-nine diet and/or VitB6

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease							
Sebastio G et al (1995);	18 CBS—	DiagnAge	EctopiaLent	Osteopor	VascDis	MentalRetard	VitB6-responsiv	Allele1	Allele2
Am J Hum	1 LG	4y	+	+	+	+	partial	G374A	G374A
Genet;	2 ON	1y	+	+	-	+(&epilepsy)	-	C770T	C770T
Federico II	3a SS	6y	+	+	-	-	+	C262T	T833C
U, Naples,	3b SR	2y	-	-	+	-	+	C262T	T833C
Italy.	3c SG	0.5y	-	-	-	-	+	C262T	T833C
	4a ML	9y	+	+	+	+	+	T833C	?
	4b MA	3y	+	+	-	-	+	T833C	?
	5 PV	29y	+	+	-	-	+	?	?
	6a DM	12y	+	+	-	-	+	C341T	?
	6b DA	10y	+	+	+	+	+	C341T	?
	7 PA	12y	+	+	+	+	+	?	?
	8 QE	25y	+	-	-	+	+	C341T	?
	9 TG	4y	+	+	+	+	-	C341T	?
	10 SA	7y	+	-	-	+(&epilepsy)	+	C341T	?
	11 TL	6y	+	+	-	+	-	?	?
	12 CG	33y	+	-	-	-	+	T833C+ins68bp	T833C
	13 MN	15y	+	+	-	-	+	T833C	T833C
	14 TR	35y	+	+	-	-	+	?	?
		« Clinical findings of 18 patients belonging to 14 unrelated families, all originating from southern Italy, are listed. Minimal criteria for CBS—were: Hcy(ine)-uria and hypermethioninemia, associated with dislocation of optic lenses. All patients met these criteria except 3b and 3c, who have not developed the ocular finding due to early VitB6 treatment. CBS activity in skin fibroblasts was tested and found to be <1% of normal values in patients 1, 2, 4a, 4b, 7, and 8.							

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease		Hcy level	Treatmnt and biochem etc contexts
Kluijtmans LAJ et al (1996); J Clin Invest	1 CBS--case	"The patient is a woman, now 20y of age, who had been admitted to the hospital at the age of 9y because of psychomotoric retardation and Marfanoid features such as excessive height, dolichostenomelia, and arachnodactyly ……At present time ie 11years after diagnosis and start of therapy, she is in a very good physical condition and her intellectual development has reached an average level. Her length is 182cm and weight 75kg. She has not any physical complaint. Ectopia lentis, osteoporosis, and vascular complications did not occur till now. (Ambiguity as to disease outcome status, due to the inconsistency of the latter two sentences, may be due to language translational difficulties. DV)….. Direct sequencing of the entire CBS cDNA revealed the presence of a homozygous G1330A transition. This mutation causes an amino acid change from aspartic acid to asparagine (D444N) in the regulatory domain of the protein….	Methionine 83uM 79uM 80uM 69uM	fHcy 178uM = >218uM tHcy 121uM = >161uM tHcy 62uM = >102uM tHcy 18uM = >58uM tHcy	None VitB6 500mg/d + Folate 5mg/day +(cumul) Betaine 6g/day
	Contr	Despite the homozygous mutation, CBS activities in extracts of cultured fibroblasts of this patient were not in the homozygous but in the heterozygous range. Furthermore, we observed no stimulation of CBS activity by S-adenosylmethionine, contrary to the 3fold stimulation in control fibroblast extract"	16-38uM	0.2-2.6uM = 10uM tHcy	normal

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Cordoba-Porras A et al (1996); J Mol Med; U Antioquia, Medellin, Columbia.	6 CBS--mean age 13y 6 Controls mean age 13y P	LDLTBARS HDLTBARS LDL%eNeg LDLOx HDLOx Met tCys 0.39nmoleq .04nmoleq 10.6% 59.6min 40.0min 440uM 55uM 0.37nmoleq .13nmoleq 8.5% 57.3min 33.5min 25uM 233uM nss nss nss nss <.05 <.05 <.05 Where: LDLTBARS = native LDL ThiobarbituricAcidReactiveSubstance, in units of nmol equivalents of MDA/mg lipoprotein protein HDLTBARS = native HDL ditto LDL%eNeg = proportion of electronegative LDL in native LDL in units of % LDLOx = susceptibility to oxidative modification of LDL and HDL measured by the kinetics of diene formation (Lag phase in units of minutes given here, they also provided maximal curve slopes in units of change in absorbance/min (not tabulated here but see next note)) HDLOx = ditto for HDL Met = methionine tCys = total Cysteine Note: the maximal curve slope of the LDL oxidative modification (not tabled here) was in agreement with the difference in lag times noted here, while that of the HDL oxidative modification was very slightly (though vnss) in disagreement with the difference in lag times noted here. Note: It is quite obvious that the ss given above have not noted any point of reference other than P<.05, and some of the ss is obviously much greater than that ie P<.001… "The patients studied were diagnosed with severe hyperhomocysteinemia on the basis of their homocysteine concentrations. The clinical information available at the time of withdrawal supported the diagnosis of classical hyperhomocysteinemia in all six patients" Note: The generally poor and easy/offhand treatment given here in the context of clinical practive initiated in that locality in the era of less stress on low-methionine diet (it may well have been somewhat in any case, there…) and cystine supplementation provides a good opportunity to view the low cysteine levels, and that in a group possibly milder in CBS deficiency than others on average.	tHcy 146uM 8uM <.05	Two CBS--treated with VitB6 + Folate, One with only VitB6 Controls approx nil treat'nt

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Mandel H et al		APCResRatio	FVLeiden	ThrombOnset	ThrombSite	ThrombTrig??	tHcy	Outcome
(1996)a; NEJM; Rambam	CBS--Family1a	?	homz/hetz	7years	deepvein, lung	diarrhea	>40uM (Hcy-uria)	Death at age 8y
Medical Center, Haifa,	CBS--Family1b	?	homz/hetz	8years	deepvein, lung brain	?	>40uM (Hcy-uria)	Death at age 13y
Israel.	CBS--Family1c	1.3	homz	8years	deepvein, lung	surgery	290uM	MRetard age 8y
Note: high degree of consanguin. in this	CBS--Family2a	2.0	hetz	7years	deepvein, brain	surgery	274uM	+MRetar age 15y
population	CBS--Family2b	3.4	none	none	none		322uM	MRetard age 17y
	CBS--Family3	2.2	none	none	none		350uM	-MRetard age 15y
	MTHFR--Family1a	?	homz/hetz	0.2years	brain	?	47uM	Death at age 2y
	MTHFR--Family1b	1.6	hetz	none	none		62uM	Healthy at 1.5y (warfarin
	MTHFR--Family2	1.6	?	0.2years	brain	diarrhea	50uM	Death at age 1.5y
	MTHFR--Family3	2.1	none	none	none		79uM	MRetard age 1y
	cbl(C/D)--	2.4	none	none	none		43uM	+MRetar age 3y
See also Quere & Lamarti (1996) imm below, and Mandel et al (1996)b imm below that		Where: APCResRatio = Activated Protein C Ratio FVLeiden = Factor V Leiden mutation ThrombOnset = Age of thrombosis onset ThrombSite = Body site of thrombosis ThrombTrig?? = Possible thrombosis trigger +MRetar = severe mental retardation MRetard = moderate/mental retardation -MRetard = mild mental retardation homz = homozygote hetz = heterozygote						
Quere I Lamarti H (1996); NEJM; St Eloi Hospital & Necker Hospital, France	15 CBS--	"We have tested for FVLeiden in 15 patients who were CBS-- (by all tests) and who were members of 13 unrelated white French Families. Among them 5 patients had deep-vein thrombosis, 2 had arterial thrombotic disease (myocardial infarction and thrombotic aortic aneurysm), and 8 were free of any vascular or thrombotic disease (age 4-28). Of the 15 patients only 3 were heterozygotes for the R506Q (FVLeiden) mutation. 2 of these 3 patients presented with venous thrombosis; the other 1 had not had thrombosis before the age of 11. Thus, 3 patients without the R506Q mutation had venous thrombosis. The activity of protein C, protein S, and Antithrombin III was normal in 2 of these 3 patients; the third could not be tested. The 2 patients presenting with arterial disease did not carry the R506Q mutation. Although the frequency of the mutation seems to be high (20%) in our population, it is very different from that reported by Mandel et al (64%), …….Since 3 of our patients without the R506Q mutation had venous thrombosis, this mutation does not appear to be a necessary requirement for thrombosis in our 13 families" Thromb- Thromb+ (no mut allele) FVLeiden++ 9 3 (one mutant allele) FVLeiden-+ 1 2						
See also Mandel et al (1996) imm above								

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Mandel H et al (1996)b; NEJM; Rambam Medical Center, Haifa, Israel. See also Mandel H et al (1996)b two imm above, and Quere & Lamarti (1996) imm above	Those of Mandel et al (1996)b two imm above, and Quere & Lamarti (1996) imm above	"The comments of Quere et al are of great interest, since they also report the occurrence of thrombosis in patients CBS-- in the absence of a coexisting FVLeiden mutation. Yet the frequency of R506Q heterozygotes in their series (20%) is 3-5 times higher than that reported among Western Europeans. They correctly point out the influence of the different consanguinity rates and genetic makeups in our two study populations, since the frequency of heterozygosity for the R506Q mutation in Arab Israeli communities is as high as 17% (unpublished data). "		
Dawson PA et al (1996); Aust NZ J Med; U Queensland Brisbane, Queensland Australia.	1 CBS--	Age Plasma: Methionine fCys tCys(derived) fHcy 3years 790uM 27uM 35uM 172uM 3years 650uM 38uM 50uM 183uM 6years 1000uM 70uM 140uM 50uM Note: Patient homozygous for G307S mutation, known to be VitB6-nonresponsive. Note: Patient 17y old in 1996, so delay in implementing betaine is possibly excusable at the date time of delay (approx 1982-85), though why methionine-restriction and cysteine-supplementation were not implemented remains cause for question….. Note: Patient had "ectopia lentis, mild mental retardation, and a number of bony abnormalities", apparently from the text sequence already at the age of 3years. Note: If the change in cysteine levels came about without any change in cysteine intake (cysteine supplementation in CBS-- becoming very poorly considered and recorded at an early stage of vitamin/betaine treatment) then this is cause for major query as to the metabolic cause of this, as excess stimulation of CBS by SAM would be expected to be pretty near maximal already given the methionine levels……	tHcy >212uM >223uM >90uM	Nil VitB6 100mg/d for 2weeks VitB6 100mg/d + Folate 5mg/day + Betaine 3g*2/day

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Van Der Molen EF et al (1997); Thromb Haemost; U Hospital St Radboud, Nijmegen, Netherlands	1 CBS—Endothe-lial Cell Line, &, >=3 Normal Cell Lines	"Endothelial cells from the human umbilical vein (HUVECs) were obtained from umbilical cords of healthy fetuses from uncomplicated pregnancies and deliveries of healthy mothers (non-smoking and without medication). Within 24h after cord collection endothelial cells were isolated by collagenase treatment according to the procedure of Jaffe at al. HUVECs were cultured under standard conditions ….our previous article. All HUVEC experiments were performed in 12-well plates with cells of 3 days in confluence in the third or fourth passage….All experiments with normal HUVECs were performed in duplo with at least 3 different cell lines. All experiments with CBS-- HUVECs were performed in triplo." The following statement by the authors ("The Hcy export and the markers of endothelial function did not differ between the control and the CBS--HUVECs under various test conditions.") is correct only with regard to the export of Hcy under only one test condition – the rest of it is very incorrect – the reason being that a simplistic, inappropriate and insufficient statistical analysis was slavishly adhered to in the face of obvious trends visible in the tabulated data (comparing only each pair of (small groups of) data within itself, and not the much larger amount of comparable data within each table). The results quite visible to me are as follows: # As they assert, across 0, 3, 30, 100nM Folinic Acid, over 24, 48, 72hours, the Hcy export did not differ between the CBS-- and Control cells, and both types of cell lines had similar increase of Hcy export over increasing time, and similar decrease (reduced to roughly only 30% at the highest dose) over increasing folinic acid. This is suggestive, in accordance with the authors, that human vascular endothelial cells do not have CBS activity, at least at that age, but this needs be considered in the light of other observations that CBS activity is low in neonates, increasing thereafter….. # Across 0, 0.3, 1, 10, 100, 1000uM Pyridoxine HCl, over 24, 48, 72hours, 13.5 times/18 the CBS-- cells exported more Hcy than the Control cells, and 4.5 times/18 the converse – this difference might have been ss….. # Across 0, 5, 10, 50, 100, 200uM Methionine, over 24, 48, 72hours, 17 times/18 the CBS-- cells exported more Hcy than the Control cells, and 1 time/18 the converse – this difference would have been ss….. # Across 0, 3, 30, 100nM Folinic Acid, over 24, 48, 72hours, the von Willebrand Factor was always (12/12) higher for the CBS-- cells than for the Control cells by an average proportion of roughly 5 or 6% – this difference would have been ss….. # Across 0, 3, 30, 100nM Folinic Acid, over 24, 48, 72hours, the Tissue Plasminogen Activator (tPA) was always (4/4) higher (rough average proportion by 20%) for the Control cells than for the CBS-- cells at 24hours, while thereafter at 48, 72hours, the Tissue Plasminogen Activator (tPA) was always (4/4 & 4/4) higher (rough average proportion by 20%, 50%) for the CBS-- cells than for the Control cells – even with multiplication of the P value by *2, *3 for the post hoc split of the group into 2 or 3 different timepoints, this difference(s) would still have been ss…. # Across 0, 3, 30, 100nM Folinic Acid, over 24, 48, 72hours, the Plasminogen Activator Inhibitor (PAI-1) had a fairly similar overall trend (4/4, 3/3, 3.5/0.5) as that of the tPA, but of less magnitude. Note: As the latter two are antagonists, it might be taken as signifying not much other than personal idiosyncrasies, but there were always at least 3 (out of an unknown total n Controls) Controls in a comparison, so….?

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Watanabe T et al (1997); J Med Invest; U Tokushima School of medicine, Tokushima, Japan.	2 CBS-- siblings:	Plasma:	Methionine	Hcy-uria	tHcy	
	Case 1, boy age 9y	Age 5days	536uM	not examined	not exam	Normal milk formula, protein intake 2.5g/ kgBW/d at age 5days and normal diet thereafter for both
		Age 19days	1,421uM	negative	72uM	
		Age 1-3months	1,500-1800uM	negative-positive	67uM	
	Case 2, girl age 7y	Age 5days	<67uM	not examined	not exam	
		2.5years	556uM	strongly positive	96uM	
	Normal		20-50uM	negative	6-10uM	
		"Case 1…..Although hypermethioninemia was detected on routine screening at 5days old, diagnosis of Hcy-uria was not made until he was 3months old because of undetectable urinary levels of non-protein bound Hcy. Hcy(ine) was detected in urine at 3months old and a marked reduction in ("markedly reduced" meant? DV) CBS activity was observed in cultured skin fibroblasts……(see table. DV)… Since this patient did not respond to high dosages of VitB6 (500mg/d*10days), a low methionine diet and betaine were initiated. His subsequent physical and psychomotor development were normal.				Low-methionin diet + betaine
		Case 2 …..However, her blood concentration of methionine was below the normal cutt-off value in mass screening at 5d old (tHcy<67uM). While her physical development was normal, she exhibited retardation, especially of speech development. At the age of 2.5y she visited Tokushima U Hospital. At that time her development quotient was 57. A test for cyanide-nitroprusside in urine was positive….(see table. DV) ….. No activity of CBS was detected in cultured skin fibroblasts. Since the patient failed to to high doses of VitB6 (500mg/d*8days), she was administered a low methionine diet and betaine. Her IQ rose from 57 at age 2.5y to 99 at age 6.5y……				Low-methionin diet + betaine
		The specific activities of methionine adenosyltransferase and betaine-homocysteine methyltransferase are lower in the fetal liver than in the adult liver, whereas the activity of 5-methyltetrahydrofolate-homocysteine methyltransferase is higher in fetal tissue than in adult tissue (cites Gaull et al 1972, 1973). This fetal enzyme pattern should direct a large portion of the available homocysteine to the 5-methyltetrahydrofolate-dependent methylation cycle rather than towards the synthesis of cystathionine, then change to an adult pattern during development. (Seems these two former abovementioned lower enzyme activities would lead to an increase in Hcy, while the latter abovementioned higher enzyme activity would lead to a decrease in Hcy?..... relative proportions of metabolic activity, as well as developmental evolution of other enzymes in these pathways need be considered also. DV). The failure to detect homocystinuria or hypermethioninemia in some newborn infants with CBS-- may therefore be explained by a quantitative difference in the activities of enzymes involved in the metabolism of methionine and Hcy for the first few weeks after birth……Most VitB6-responsive CBS-- are missed on newborn screening, because the blood level of methionine is not sufficiently elevated in the plasma during the first few days of life. However our patients did not have a VitB6-responsive form of CBS--. ….. Poor protein intake can be excluded as a cause in our patient. Therefore we suggest that the activities of enzymes other than CBS in methionine metabolism probably differed in the neonatal period in these patients. Thus genotype may not always be consistent with phenotype in patients with inherited diseases.				

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Surtees R et al (1997);			Gly	Ser	5-MTHF	Methionine	S-Ado-Met	tHcy	
Pediatr Res; Institute of Child Health, London, UK	5 CBS--age 6-14y, VitB6-non-responsiv	CSF beforeBet:	4.8uM	23uM	.050uM	35uM	0.25uM	1.2uM	2 on low-Met diet + Folate
		Stat Signif:	ss vsRR			ss vsRR		ss vsBoth	
		CSF afterBet:	3.3uM	34uM	.031uM	78uM	0.38uM	0.32uM	Ditto + + Betaine 250mg/ kgBW/d * 3-6month
		Stat Signif:	ss vsRR			ss vsRR	ss vsRR	ss vsBoth	
	Normals, age-matched (0.4-12.5y) but to who?...	Ref Range:	7.8uM	61uM	.061uM	4uM	0.21uM	<0.10uM	
		P(ANOVA):	<.001	.001	.08	<.001	.006	<.001	
	10 CBS--Including the above 5, age otherwise unknown, VitB6-responsiv otherwise unknown	Plasma beforeBet:	272uM	110uM		275uM		99uM	3 on low-Met diet + Folate
		Stat Signif:				ss vsRR		ss vsBoth	
		Plasma after Bet:	178uM	161uM		275uM		43uM	Ditto + + Betaine 250mg/ kgBW/d * 3-6month
		Stat Signif:	ss vsbB	ss vsbB		ss vsRR		ss vsBoth	
	Normals, age-matched (0.4-12.5y) but to who?...	Ref Range:	221uM	130uM		21uM		6uM	
		P(ANOVA):	.006	.009		<.001		<.001	

Where:
CFS = CerebroSpinal Fluid, Gly = Glycine, Ser = Serine,
5-MTHF = 5-methyl-tetrahydrofolate,
S-Ado-Met = S-Adenosyl-Methionine,
Stat Signif = Statistical Significance,
ss = statistically significant (and see note below),
vsRR = versus Reference Range, vsbB = versus before Betaine
vsBoth = versus both the other two values,

Note: "Post hoc significance testing used Tukey's HSD test; P values <.05 were regarded as significant. Because of the relatively small number of CFS samples, these results were also analysed using nonparametric statistical methods (….analogous. DV)."

"The primate brain normally contains CBS (cites Rassin et al 1981), and its deficiency would be expected to lead to an accumulation of Hcy within the cells of the CNS. In other tissues, intracellular accumulation of Hcy causes export of Hcy into the extracellular space. In the CNS, CSF is in continuity with the extracellular space, and accumulation of Hcy in this fluid is likely to reflect accumulation within the brain. The mammalian brain does not contain the enzyme betaine-homocysteine methyltransferase (cites McKeever et al 1991). So the effect of betaine is most probably mediated through changes in the plasma Hcy concentration. Because we found that CSF Hcy concentrations decreased with betaine treatment, it suggests that the reduction in plasma tHcy caused by betaine is responsible for the decrease in CSF tHcy. It is possible that a reduction in plasma concentration of Hcy facilitates the active removal of Hcy fthe brain, thereby reducing its accumulation in the CFS…..Another potential mechanism might involve oxidation of Hcy to homocysteid acid, which is an excitotoxin that activates the glutamate N-methyl-D-aspartate receptor (cites Cuenod et al 1990)."

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Wilcken DEL Wilcken B (1997); J Inher Metab Dis; Prince Henry, Prince of Wales, and New Children's Hospitals, Sydney, NSW, Australia	40 CBS--, 17 VitB6-responsiv 15 VitB6-nonrespon Age 30, (9-66) years 8 VitB6?? Age??	"Patients and Methods The study population comprises 40 patients from 25 sibships for whom there is long-term follow-up with known outcomes. 3 recently diagnosed patients are not included. The patients were diagnosed on the basis of characteristic and clinical features plus elevated levels of plasma methionine and free homocyst(e)ine with low free cyst(e)ine. All patients had elevated urinary homocystine. …… Treatment Regimens All treated patients received pyridoxine 100-200mg/day, folic acid 5mg/day, and most had intermittent hydroxocobalamin by injection according to the serum VitB12 levelmeasured usually twice-yearly. Pyridoxine nonresponsive patients all received in addition 6-9g/day oral trimethylglycine (betaine) given in two divided doses. One pyridoxine-responsive patient also received betaine and this was maintained throughout her subsequent pregnancy. Diet was not closely monitored, but general advice was given to reduce the intake of foods with high methionine content. During the last 4years VitB12 therapy 1mg by intramuscular injection every 1-3months has been given to pyridoxine-nonresponsive patients irrespective of their serum VitB12 levels. (Note: The high detail of this description suggests that cysteine supplementation was in fact not implemented, rather than merely not mentioned. DV) Vascular Events Vascular events were documented in surviving and nonsurviving patients in relation to whether or not they were receiving treatment at the time of the event, and to what the treatment was. We assessed the effect of treatment on the occurrence of vascular events by comparing the findings in our treated patients with those in the untreated patients reviewed by Mudd and Colleagues (1985). They found that at the time of maximum risk (beyond the age of 10y) there was 1 event per 25y. We compared our risk data with that statistic. This is a conservative estimate since, before treatment started, 2 patients had already had vascular events, which enhances the risk in untreated patients to about 1 event in 10y (Mudd et al 1985). (Note: this "enhancement" might only apply if a patient doesn't know why the event occurred, or what to do about it – otherwise it might not, and might even decrease risk by fright value and a resulting increase in treatment compliance…DV) Results In the 40 patients there were 10 deaths at ages 2-30y. …only 2 were receiving effective treatment at the time of death, and only 1 of the 2 was CBS---related, …massive pulmonary embolus. (Note: There were 2 deaths whilst on "No effective treatment" for which no further details are provided. DV). Of the 32 patients receiving effective treatment, 17 were pyridoxine-responsive. During 281 patient years of treatment which maintained plasma total free homocyst(e)ine consistently <20uM, there were 2 vascular events in this group. 1 patient, referred to above, succumbed from a pulmonary embolus and a second patient, who had had 4 vascular events prior to starting treatment at age 41y, subsequently had a myocardial infarction at the age of 55y. From the data of Mudd et al (1985), untreated, 11 events would have been expected, relative risk =0.17 (CI .04-0.80) P = .026. During 258 patient-years of treatment I the 15 pyridoxine-non-responsive patients, no vascular event occurred. These 15 nonresponsive patients have current mean+/-SD total free homocyst(e)ine of 33+/-17uM. From the data of Mudd et al (1985), in untreated nonresponsive patients at least 10 events would have been expected, P = .005. 19 patients of the series had a total of 19 major and 15 minor operatios requiring an anaesthetic and there were 3 successful pregnancies, one in a patient receiving betaine. There were no thromboembolic complications. Thus, during 539 patient-years of treatment in all 32 patients there were 2 vascular events, whereas, if untreated, 21 events would have been expected. These findings indicate that treatment markedly reduced the relative risk to .09 (95%CI .02-0.38) P = .0001." (Continues)	tHcy very roughly <40uM tHcy very roughly 66uM
(Continues)		(Continues)	

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(Continued) Wilcken DEL Wilcken B (1997); J Inher Metab Dis; Prince Henry, Prince of Wales, and New Children's Hospitals, Sydney, NSW, Australia	(Contin)	(Continued) Now, as Mudd et al (1985)'s risk of thrombosis is not constant over time, but rather sigmoidal, with the risk at <10y of age and >32y of age being approximately half the risk at (max) age 21, and as the average ages of the subgroups of pyridoxine-responsive(-nonresponsive) have not been provided here by W & W, the only comparison that may be properly attempted is that between W & W's combined (VitB6-responsive(-nonresponsive)) group and Mudd et al's combined group. That of the sentence immediately preceeding this paragraph. Now, as Mudd et al's statistic of 1 event per 25y refers to (both of, approximately equal) the respective periods of maximum risk of thrombosis of the VitB6-responsive(-nonresponsive) subgroups (ages 22y and 15y respectively, mean 18.5y), and as the average age of W & W's combined group is 32y, then their group here is expected to be a nontrivialy lower risk group, and therefore their estimation of relative risk is not conservative, but a rather a nontrivial overestimation/inference of treatment efficacy. Furthermore, here, as in Mudd et al the oldest cases are milder cases by survival, but their oldest cases here will be milder cases than Mudd et al's due to (hopefully…) improved diagnosis. Also, their youngest cases here will too be milder cases than Mudd et al's due to (hopefully…) improved diagnosis. Therefore the real relative risk is likely to be nontrivialy higher (less treatment efficacy) than they report here, possibly so as to include unity in the CI, though note that given the lowish n, the suggestion of efficacy is certainly strong enough to warrant continuing with the treatment, while considering possible improvements (ie earlier detection, cysteine supplements, VitC, VitE, and better dietary achievements?....)	
Bonham JR et al (1997); J Inher Metab Dis; U Wales College of Medicine, Cardiff, UK	12 CBS--, 13 CBS-+ Obligate Heterozyg 22 Normal	"We have compared the relationship between total homocysteine and free homocystine in plasma in 12 patients with CBS—over a 2year period, 13 obligate heterozygotes and 22 normal individuals. The correlation of free and total homocystine shows a tri-phasic distribution: (i) an increase in free homocystine could not be detected reliably when the total homocysteine was less than 60uM (ii) an increase in free homocystine between <1 and 20uM correlated with a linear but more pronounced increase in total homocysteine ranging from 60-150uM, indicating some binding of free homocystine to components in plasma (iii) when free homocystine exceeded 20uM there was an equal increase in total homocysteine in the range of 150-250uM, suggesting that the plasma binding capacity for homocystine had been exceeded." "A patient assessed at a time of poor control (tHcy 160uM, free homocystine 20uM) showed a marked impairment in endothelium dependent brachial artery dilation compared to age/sex matched healthy subjects (n = 7, -.08mm vs +0.20mm). Following a short period of better control (tHcy 35uM, free homocystine <1uM) the response remained impaired (+.01mm), which suggests that even modest hyperhomocystinemia in these patients may be clinically important. It seems likely that increased tHcy in the range 30-60uM may be important and that measurement of free homocystine may be an inadequate means of monitoring patients in this range"	Triphasic Hcy pooling: tHcy <60uM & fHcy(ine) <1 tHcy 60-150uM & fHcy(ine) 1-20uM tHcy 150+xuM & fHcy(ine) 20+xuM

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Authors, Journal, Site	Study Group	Disease	Hcy level
Lobo CA, Millward SF (1997); J Vasc Interv Radiol; Ottowa Civic Hospital, Ottowa, Canada	1 CBS--??	Female aged 34 in 1990, hypercholesterolemia, 12-pack-year smoking history, hypertension for 17years, no diabetes, Oct 1989 left hemispheric stroke, May 1990 admitted with 2week history of painful and discoloured left big toe, angiographic 2cm 50% stenosis of left popliteal artery…..percutaneous transluminal angioplasty, anticoagulation not continued post-op, antiplatelet therapy not initiated, 1day post-op thrombosis of popliteal artery and of tibioperoneal trunk, "….intraarterial streptokinase infusion. This resulted in successful recanalization of the popliteal artery with no residual stenosis or thrombosis but only the anterior tibial artery was still patent, the tibioperoneal trunk was still occluded. The streptokinase was discontinued and the intravenous heparin was initially continued; then anticoagulation was continued with oral warfarin (5mg/d), although no antiplatelet treatment was initiated." 2Weeks later deterioration, rest pain, open surgical thrombectomy of the popliteal artery and tibioperoneal trunk, 4weeks later recurrent rest pain, angiographic complete occlusion of superficial femoral and popliteal arteries with reconstitution of anterior tibial artery, pre-op Ancrod andti-fibrinogen, common femoral to anterior tibial artery reverse saphenous vein graft placed, post-op warfarin 5mg/d…. 3weeks later recurrent rest pain, leg critically ischemic, black eschar along calf incision, no distal pulses left leg, amputation, pathology: arterial and venous thrombosis with no underlying pathology and only minimal atherosclerosis, oral warfarin discontinued, 1992 "3 pulmonary emboli and a myocardial infarction. Great difficulty was experienced in controlling the degree of anticoagulation with warfarin; therefore treatment with a continuous subcutaneous heparin infusion was initiated. April 1995, it was noted that she had developed osteoporosis, which was thought to be secondary to her long history of heparin use. A hickman catheter was inserted for Ancrod infusion. After insertion of this catheter, she developed thrombosis of the superior vena cava. A subcutaneous catheter was inserted for injections of low-molecular-weight heparin 15,000 U twice daily." November 1996, she suffered a small right hemispheric stroke, all normal lab: antithrombinIII, anticardiolipin antibody, lupus anticoagulant, antinuclear antibody, rheumatoid factor, antigenic protein C, functional protein C, free protein S, C3, C4, …….tHcy 58uM and >80uM, would not consent to further testing, so etiology? "Nevertheless, she was subsequently treated with VitB6 (pyridoxine) 100mg/day, VitB12 500ug/day, and folate 1mg/day and she continued to receive subcutaneous low-molecular-weight heparin 5000U*2/day. No further thrombotic events have occurred after 1year of follow-up"	tHcy 58uM & >80uM
Naughten ER et al (1998); Eur J Pediatr; World reviewed	CBS--screening programs & epidemiol inferences world-wide	"Abstract Newborn screening for CBS-- (homocystinuria; HCU) was started in the late 1960s using a bacterial inhibition assay (BIA). At least 7 countries have either national or regional screening programmes; 12 programmes are known to have discontinued. The wrldwide incidence of HCU is approximately 1 in 335,000 but varies from 1:65,000 (Ireland) to 1:900,000 (Japan). Methodologies include the BIA, one-dimensional or thin-layer amino acid chromatography and, more recently, tandem mass spectrometry. The BIA diagnostic cut off concentration of blood methionine varies from 67 to 270uM (10-40mg/l) with a median of 135uM (20mg/l). In Ireland, 25 cases of HCU from 19 families have been identified from 1.58 million newborn infants since 1971; 21 cases were detected through the screening programme. Of the 4 missed cases, 3 were breast-fed (= lower-methionine diet. DV) at the time of blood collection and 1 was pyridoxine responsive. These findings are in broad agreement with the results from 5 other programmes, in which approximately 1 in every 5 cases was missed by the screening programme. Early hospital discharge, low protein intake, high blood methionine cut-off concentration and pyridoxine responsiveness were all identified as contributing to missed cases."	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level
Yap S, Naughten E (1998); J Inher Metab Dis; The Children's Hospital, Dublin Ireland	All 25 CBS--detected in Ireland 1971-96. 12 females, 19 families. 24/25 VitB6-non-responsiv	"Subjects: This is a retrospective study of all 25 cases of HCU detected in Ireland between 1971 and 1996 either by the national newborn screening programme or by clinical presentation. The data were extracted from the clinical notes. 21 cases were identified by the national screening programme. Of the remaining 4 cases missed on screening, 3 were breast-fed and 1 is pyridoxine responsive. All but one were started on treatment at diagnosis…. Of the 4 cases missed on screening, 3 presented with ectopia lentis after the age of 2years…. All 21 cases of HCU detected by newborn screening had high blood methionine concentration of more than 100uM, assayed by the bacterial inhibition assay (BIA) on the heel prick blood sample taken on day3 to day5 of life. A liquid blood sample, separated and deproteinized within 10min of collection, was requested from these infants and assayed using an amino acid analyser for methionine, free homocystine and cystine. A high blood methionine and free homocystine with a low cystine confirmed the diagnosis for those detected on newborn screening and those that presented clinically…. Clinical management and follow-up: All patients diagnosed as having HCU had their pyridoxine status (pyridoxine-responsivity status meant. DV) determined clinically by commencement on oral pyridoxine 50mg*3/day as inpatients….Pyridoxine responsiveness was indicated by a rapidly falling methionine level and clearing of free homocystine from the plasma, while pyridoxine nonresponsiveness was indicated by persistently high or rising plasma methionine and free homocystine. In pyridoxine-nonresponsive patients, dietary management was commenced by restricting dietary methionine and using a methionine-free, cystine-supplemented synthetic mixture. Methionine was added as a proprietary food preparation or as breast milk and the amount was titrated against the plasma methionine and free homocystine concentration. Two-thirds of the total protein intake was derived from the synthetic methionine-free, cystine-supplemted mixture and the remaining third from the natural methionine-containing foods (P. Howard, personal communication). Plasma VitB12 and folate were assayed and, if they were found deficient, the patient was given supplements. Once stabilized, blood was drawn, deproteinized, and analysed for methionine, free homocystine and cystine at least once a month, or more frequently if clinically indicated, to monitor biochemical control. During all episodes of acute illness, prompt treatment of the illness with additional supportive care, including adequate hydration and aspirin or dipyridamole, was given to prevent blood stasis….. Upon discharge from hospital, patients initially attended the outpatient department fortnightly and thereafter at 4- to 6-weekly intervals. They were reviewed minimally at least 4 times per year. At every clinic visit, growth parameters were measured, a general physical examination was carried out by a doctor, and blood was drawn, deproteinized and analysed for methionine, free homocystine and cystine to monitor biochemical control. Experienced dieticians… Annual detailed ophthalmological examination….. Initially, skeletal radiology was performed yearly….. Up to 1990, annual clinical cardiovascular assessments…… IQ assessments were performed at 2- to 6-yearly intervals. Psychological support was provided whenever necessary by the clinical psychologist. Results: "3 patients who were detected by screening developed complications due to non-compliance with the prescribed diet, as indicated by dietary history and poor biochemical control are in Group 2. The 4 cases missed on screening presented with complications after 2years and are in Group 3. 1 of the 4 cases missed on screening and never treated was recently referred to our clinic aged 21years with recognized complications of untreated HCU. She had bilateral ectopia lentis and optic atrophy, mental retardation, osteoporosis, dolichostenomelia (arm span 164cm, height 163cm) but no documented evidence of thromboembolic events." (And see also the second of the following tables) (Continues)	

(Continues)

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal,	Study Group	Disease	Hcy level
(Continued) Yap S, Naughten E (1998); J Inher Metab Dis; The Children's Hospital, Dublin Ireland	(Contin)	(Continued)	tHcy

	Age	AgeTx	nPunct	meanMetM	meanMetL	meanMetH	meantHcyL	meantHcyH	meanHcM
Group 1 n = 18	14y (2.5-23)	19d (7-42)	58punct (19-98)	73uM (47-134)	16uM (1-35)	348uM (76-762)	<60uM (?)	178uM (136-302)	104uM, (78-178)
Group 2 n = 3	17y (12-23)	21d (9-42)	82punct (63-101)	78uM (70-85)	17uM (4-43)	452uM (161-789)	91uM (<60-154)	225uM (211-238)	169uM (141-188)
Group 3 n = 4 or 3	18y (17-21)	4y (2.4-7)	60punct (30-94)	60uM (50-67)	21uM (3-36)	353uM (116-791)	<60uM (?)	172uM (157-192)	87uM (80-98)

All 25 CBS--detected in Ireland 1971-96. 12 females, 19 families. 24/25 VitB6-non-responsiv

Where:

Group 1 were detected on screening; on diet; no complications,
Group 2 were detected on screening; noncompliant (whole life, whole life, last 6.5 of 23years) to diet; with corresponding complications (osteoporosis + lowIQ, ectopia lentis + lowIQ, iridodonesis, respectively)
Group 3 were missed on screening and presenting subsequently with complications (3* ectopia lentis)

Age = age at survey, AgeTx = age at commencement of treatment,

nPunct = the minimum number of venupunctures experienced consistent with data reported over treatment period surveyed (ie the maximum of the number of methionine, or Hcy, (which were close to each other though not always equal) measures in each individual's data),

meanMetM = the mean of each Group (1,2,3) of individual patients lifetime median plasma methionine,
meanMetL = the mean of each Group (1,2,3) of individual patients lifetime range Lowest value of plasma methionine
meanMetH = the mean of each Group (1,2,3) of individual patients lifetime range Highest value of plasma methionine

meantHcyL = the mean of each Group (1,2,3) of individual patients lifetime median plasma tHcy
meantHcyH = the mean of each Group (1,2,3) of individual patients lifetime range Lowest value of plasma tHcy
meanHcM = the mean of each Group (1,2,3) of individual patients lifetime range Highest value of plasma tHcy
(Where tHcy was derived using the relationship of fHcy(ine) (data values provided by Yap and Naughten here) to tHcy reported by Bonham et al (1997), such that:
tHcy = 60 + 4.5(fHcy(ine)) for values of fHcy(ine) < 20uM, and
tHcy = 60 + 90 + (fHcy(ine) – 20) for values of tHcy(ine) >=21uM

		Detected by Screening		Missed by Screening
	Total	Group 1	Group2	Group 3
Tot n Detected	25	18	3	4
Ectopia Lentis	6	0	2	4
Osteoporosis	2	0	1	1
Mental Handicap	4	0	2	2
Thromboembolism	0	0	0	0

"Increasing myopia may be the first sign of persistent poor compliance, prior to ectopia lentis (Burke et al 1989), as seen in 3 cases in Group 1 with high lifetime medians of free homocysteine levels of 18, 18, and 48uM, despite their insistence on good dietary compliance."

"Mudd et al (1985), in a large HCU group, reported that the chance of suffering a clinically detected thromboembolic event event for the untreated pyridoxine-nonresponsive patient is 25% by the age of 16years and 50% by 29years, with the maximal likelihood of the event occurring between 12.5 and 17.5y of age (Confirmed. DV). None of the patients reported in this study developed detectable thromboembolic events." (Continues)

(Continues)

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level
(Continued) Yap S, Naughten E (1998); J Inher Metab Dis; The Children's Hospital, Dublin Ireland	All 25 CBS-- detected in Ireland 1971-96. 12 females, 19 families. 24/25 VitB6-non-responsiv	Now, given that the CBS-- cases here are very nearly all (24/25) VitB6-nonresponsive, the comparison with Mudd et al (1985)'s data for untreated outcomes is relatively straightforward compared to the analogous comparison of Wilcken and Wilcken (1997)'s outcome data with Mudd et al's data – as the average age of Group 1 and Group 3 combined (n = 18 + 4 = 22) is 15years (this includes the untreated years of group 3) , at least 5 thromboembolic events would have been expected in the absence of treatment, and the difference between 0 and 5 events is roughly ss at the 95% confidence level. Now, although it may certainly not be said that the treatment here could have been statistically significantly more efficacious than that of the Australian CBS-- cases repoted on by Wilcken and Wilcken, there is a substantial suggestion that it may have been in regard to thromboembolism, on taking into account that there has been far more venupuncture (which has been documented as a precipitant of thromboembolism, which theoretical considerations strongly support) in the Irish cases of Yap and Naughten (an average of a minimum of 58 punctures each) than in the Australian cases of W & W, and that the increased sulfate provided by the cystine supplementation plausibly increases the sulfation of ie heparin to an extent that increases its ability to prevent thrombosis (CITATION). The obvious implication being that increased Hcy is not the only metabolite level change that is pathogenic in CBS--. Noteworthy also is the apparently scrupulous attention to establishing the low-methionine, cystine-supplemented dietary treatment at a very early stage of infancy, and following through with comprehensive reinforcement, for which the practitioners ought be highly commended. However, note that even so, some ammount of non-compliance is likely to creep in as time passes, and that in this regard, it is likely that the cystine supplementation would suffer less non-compliance than the low-methionine diet. Overall, there is a fairly strong suggestion, that all CBS--treatment regimes should include cystine supplementation – which many apparently do not. Note that CBS-- cohorts have not yet been followed into old age, where factor suboptimality is manifest in increasingly worse outcomes, in general. Ps (the absence of the plasma cystine/cysteine values from the data of Y & N???)	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level
Walter JH et al (1998); Eur J Pediatr; Royal Manchester Children's Hospital, Manchester, UK.	All 31 CBS-- diagnosed /managed since 1962	Since 1962 we have been responsible for the diagnosis and/or the management of 31 patients with CBS--, 23 of whom have been non responsive to pyridoxine. 12 patients, all of whom are non responsive, were diagnosed after an initial finding of raised methionine on our newborn screening programme (started in 1969). The eldest patient from this group is now 25y of age. All other patients (10 non responsive, 8 responsive presented with typical clinical features of homocystinuria. Patients non responsive to pyridoxine have been assessed 3-monthly and those with pyridoxine responsive disease yearly. On each occasion blood has been collected, deproteinised and assayed with an amino acid analyser. The concentration of homocystine measured in this way represented plasma free homocyst(e)ine…. In our patients only 8 of 31 were pyridoxine responsive, presumably due to a different genetic population and also to to ascertainment bias as a consequence of our newborn screening. Following a diagnosis of CBS--, patients should be treated with VitB6 at a dose of at least 500mg/day for a period of several weeks to determine their response. Folic acid requirements are probably higher in CBS-- as a consequence of increased flux through the remethylation pathway, and lack of folate may lead to a suboptimal response to treatment with pyridoxine (cites Wilcken and Turner 1973). Patients should therefore be treated with folic acid (5mg/day) in addition to pyridoxine. Although large doses of pyridoxine may be associated with the development of peripheral neuropathy this has not been a complication in our patients (cites Mpofu et al 1991)……. Because raised methionine is not prominent in newborn infants with pyridoxine responsive disease this type of homocystinuria is rarely diagnosed by newborn screening. The diagnosis is consequently made only after the development of complications which are largely irreversible. In our experience, however, the skeletal, neurological and vascular problems associated with homocystinuria have not progressed following treatment with pyridoxine. The median IQ for our patients with pyridoxine responsive disease was 82 with a range of 57-101. There was no fall in IQ after treatment had been started. Eye disease, however, if advanced, may lead to glaucoma and further damage…….. Dietary restriction of methionine, by limiting natural protein intake, necessitates the use of an amino acid supplement, to provide other essential amino acids, vitamins minerals and trace elements and special low protein foods to provide sufficient caloric intake (This might not be correct – limitation of protein to the physiological minimum on the bases of the essential amino acids other than methionine, in the context of choosing those food protein sources that are relatively lower in methionine, might not require the use of any supplemental amino acids other than cysteine (However the use supplemental cysteine can be applied to reduce the amount of methionine, which is largely thereby substitutable, required in the diet, ie reduction of the methione intake to a level so low that other essential amino acids then require supplementation…CHECK THIS against the earlier good works examining this, and food tables). Furthermore, there is nothing "special" about fruit, or vegetable oils, which in general can meet caloric requirements with little addition to the protein (and somewhat particularly methionine) intake. DV). Cysteine becomes an essential amino acid in CBS-- and needs to be provided in an amino acid supplement. The practicalities of the diet are similar to phenylketonuria. We use a protein exchange system in both conditions but in CBS-- one exchange contains approximately 20mg methionine, rather than 50mg of phenylalanine. The daily amount of methionine tolerated in order to achieve good control (plasma free homocyst(e)ine < 10uM) varies somewhat between patients but is relatively constant throughout life. In the 23 patients treated in our clinic methionine has been restricted to a median value of 230mg/day with a range of 160-900mg. Many children, particularly adolescents and those diagnosed after infancy, find the low methionine diet difficult. Problems with compliance are common, and an increase in natural protein intake is rapidly followed by a rise in plasma homocyst(e)ine levels. Reduced intake of the amino acid supplement may also be associated with raised homocyst(e)ine levels and poor growth due to protein catabolism……	Equates to tHcy <50uM

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level
(Continued) Walter JH et al (1998); Eur J Pediatr; Royal Manchester Children's Hospital, Manchester, UK	(contin) All 31 CBS-- diagnosed /managed since 1962	(Continued) We have used betaine in 13 of our patients with non pyridoxine responsive CBS-- (250mg/day two to three divided doses). In the majority of patients methionine levels increased significantly (before treatment: mean plasma methionine 83 uM, after treatment: mean 295uM, P = .004) although, as a group, biochemical control, as assessed by a fall in plasma free homocyst(e)ine, was not improved (before treatment: mean plasma free homocyst(e)ine 35uM, after treatment: mean 33uM, P = 0.89). Compliance with betaine in many cases was poor, and this probably explains the lack of efficacy (Their reasoning here seems dubious – it might be at least as likely that compliance with the low-methionine diet decreased, in response to a perception that with the additional treatment, less stringent adherence to the diet would be necessary….assuming the amino acid supplement was easy enough to comply with?.....DV)….. None of our 11 patients diagnosed on newborn screening developed physical stigmata of homocustinuria during childhood. The median IQ for this group of patients was 100 with a range of 84-117. This is significantly better than that found in patients diagnosed after infancy (pyridoxine non responsive patients diagnosed after infancy: median IQ 58, range 20-86, P<.0001; pyridoxine responsive patients diagnosed after infancy: median IQ 82, range 57-101, P = .02). During adolescence control in some individuals deteriorated. One patient suffered from fainting episodes, numbness and weakness of the left arm and leg from the age of 17, although a brain scan and angiography were reported as normal. Despite these symptoms she failed to comply with diet or take regular pyridoxine. At 21y of age she was found on ophthalmological review to have developed iridodonesis and phacodonesis, with backward displacement of both crystalline lenses leading to a progressive myopia. Another patient whose control had deteriorated had a respiratory arrest and died at the age of 19y following a short history of headache and increasing confusion. At post mortem he was found to have an extensive thrombosis of the superior sagittal sinus and transverse and lateral sinus. There was, however, no evidence of atheroma in the cerebral arteries. The outcome for patients with pyridoxine non responsive disease diagnosed after infancy was poor with a median IQ of 58, range 20-86. Two siblings diagnosed shortly after the original description of homocystinuria at the ages of 7 and 13y were unable to comply with treatment and died in childhood from pulmonary embolism and thrombosis…. The clinical manifestations of the disease can be avoided with appropriate therapy but only in those children treated from soon after birth. Unfortunately patients with pyridoxine responsive disease rarely have a sufficiently raised methionine in the first weeks of life to be detected on current screening programmes. As a consequence treatment is most often started in patients with pyridoxine responsive disease only after the development of irreversible damage. Although pyridoxine, with the addition of folic acid, prevents further deterioration in patients with pyridoxine responsive CBS--, a better outcome for such patients will only be achieved if they are identified earlier in life. More sensitive newborn screening methods will need to be developed that are able to detect pyridoxine responsive disease. In non responsive patients a low methionine diet with amino acid, mineral and vitamin supplements can be highly successful treatment. However, homocyst(e)ine levels must be kept throughout life, as a loss of good biochemical control at any age is associated with the development of serious complications which may be life-threatening." Note: It may well be that if response to VitB6 **alone** is not sufficient to decrease Hcy levels to very near normal, and if VitB6 is **not used alone** in **any case**, that it may be desirable to incorporate cystiene supplementation in treatment (in order to ensure more optimal amounts of **each of** cysteine and sulfate to be available for their respective biological roles), due to decreased production through the CBS pathway. This desirability of cysteine supplementation may also apply in the case of suboptimal compliance even when VitB6 **alone** is sufficient to decrease Hcy levels to very near normal, and VitB6 is **used alone.** (Continues)	Equates to tHcy >75uM, >73uM
(Continues)			

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal,	Study Group	Disease	Hcy level
(Continued) Walter JH et al (1998); Eur J Pediatr; Royal Manchester Children's Hospital, Manchester, UK	(contin) All 31 CBS--diagnosed /managed since 1962	(Continued) Note: The Groups in the table above have been ordered and numbered so as to correspond (1, 2, 3) with those of Yap and Naughten (1998) of the study immediately above, and so facilitate comparison. Age AgeTx nPunct Myop EctLent Irid DevD Ep Marf/Arach/Scol CVA Group 1 n = 11: 15y (2-25) <1m 60 0 0 0 0 0 0 0 Group 2 n = 2: 20y (19-22) <1m <?80 1 1 1 0 0 0 1or2? (>=1 thromb) Group 3 n = 10: 25y (12-39) 10y (3-32) 60 4 7 >7 8 3 4 2 (2 thromb) Group 4 n = 8: 37y (27-48) 16y (9-29) 21 4 6 7 2 1 3 1 (? thromb) Where: All 31 CBS--diagnosed /managed since 1962 Group 1 VitB6-nonresponsives detected on newborn screening; on diet with cysteine etc suppl; no complications, Group 2 VitB6-nonresponsives detected on newborn screening; noncompliant (beginning from adolescence in both) to diet with cysteine etc suppl; with corresponding complications (as tabled) Group 3 VitB6-nonresponsives missed on newborn screening and presenting subsequently (treatment as noted) with complications (as tabled) Group 4 VitB6-responsives missed on newborn screening and presenting subsequently (treatment VitB6 + Folate) with complications (as tabled) Age = age at survey, AgeTx = age at commencement of treatment, nPunct = the approximate number of venupunctures derived from the description of the monitoring schedule Myop = Myopia, EctLent = Ectopia Lentis, Irid = Iridodonesis DevD = Developmental Delay, Ep = Epilepsy or Salaam (infantile) spasms Marf/Arach/Scol = Marfan Syndrome/ Arachnodactyly/ Scoliosis CVA = CardioVascularAccident Now, Mudd et al (1985), reported that the chance of suffering a clinically detected thromboembolic event event for the untreated pyridoxine-nonresponsive patient is 25% by the age of 16years and 50% by 29years, with the maximal likelihood of the event occurring between 12.5 and 17.5y of age. (See Mudd et al (1985) ( ) above, and in print, for greater detail.) So, on that basis, Group 1 if untreated would have been expected to have suffered approx 2 thromboembolic events, but suffered 0 events, which would be a nss (potentially due to insufficient n) difference. Likewise, Group 2 would have been expected to have suffered <1 thromboembolic event during the treated period (assume up to adolescence at 13years), and suffered 0 events. And, during the untreated period would have been expected to suffer <1 thromboembolic event but suffered >=1 event. Differences would be nss on the basis of insufficient n alone. Likewise, Group 3 would have been expected to have suffered approx 2 thromboembolic events during the untreated (assuming perfect compliance for the 8 non-fatal cases) period of their lives, and experienced 2 (fatal) events. And, during the treated period (n =8) (following diagnosis), if untreated would have been expected to have had approx 2 thromboembolic events, but had 0 events. Differences would be nss on the basis of insufficient n alone. Likewise, Group 4 would have been expected to have suffered approx 1 thromboembolic event during the untreated period of their lives, and experienced ?(<=1) event. And, during the treated period (following diagnosis), if untreated would have been expected to have had approx 3 thromboembolic events, but had ?(<=1) events. There was only 1 CVA noted and the report did not allow this to be allocated to either pre- or post-diagnosis/treatment periods, hence the "?". Differences would be nss on the basis of insufficient n alone.	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level
(Continued) Walter JH et al (1998); Eur J Pediatr; Royal Manchester Children's Hospital, Manchester, UK	(contin) All 31 CBS-- diagnosed /managed since 1962	(Continued) Now, here, as in Mudd et al (1985) the oldest cases are milder cases by survival, but their oldest cases here will be milder cases than Mudd et al's due to (hopefully…) improved diagnosis. Therefore the real relative risk is likely to be (trivialy?) slightly(?) higher (less treatment efficacy) than implied here, increasing the noted probability that unity would be included in any CI for any comparisons of Groups 1, 3 or 4 with Mudd et al's outcomes report, though as noted given the lowish n, the suggestion of efficacy is certainly strong enough to warrant continuing with the treatment, while considering possible improvements (ie earlier detection, , VitC, VitE, and better dietary and cysteine supplementation achievements?....) Now, although it may certainly not be said that the treatment here could have been statistically significantly more efficacious than that of the Australian CBS-- cases repoted on by Wilcken and Wilcken, there is a substantial suggestion that it may have been in regard to thromboembolism, particularly in the case of the VitB6-nonresponsive cases. On taking into account that there has been far more venupuncture (which has been documented as a precipitant of thromboembolism, which theoretical considerations strongly support) in the English cases of Walter et al (an average of a >60 punctures each) than in the Australian cases of Wilcken & Wilcken (1997) ( ) above, and that the increased sulfate provided by the cystine supplementation plausibly increases the sulfation of ie heparin to an extent that increases its ability to prevent thrombosis (CITATION). The obvious implication being that increased Hcy is not the only metabolite level change that is pathogenic in CBS--. Noteworthy here, similarly to as in Yap and Naughten (1998) ( ) above, is the (though somewhat less scrupulous?) attention to establishing the low-methionine, cystine-supplemented dietary treatment at a very early stage of infancy, and following through with reinforcement. However, some ammount of non-compliance is likely to creep in as time passes, and that in this regard, it is likely that the cystine supplementation would suffer less non-compliance than the low-methionine diet. Overall, there is a fairly strong suggestion, that all CBS--treatment regimes should include cystine supplementation – which many apparently do not. Note that CBS-- cohorts have not yet been followed into old age, where factor suboptimality is manifest in increasingly worse outcomes, in general.	
Kluijtmans LAJ et al (1998); Blood; U Hospital Nijmegen, Nijmegen, The Netherlands (Continues)	24 CBS-- Dutch cases, 23/24 VitB6-responsiv, Possibly not all of Dutch CBS-- cases known at the time.	"Patients. 24 patients, 14 men and 10 women from 18 unrelated kindereds, with homocystinuria caused by CBS-- were studied for FVL and the MTHFR 677C>T polymorphism. 23 patients were pyridoxine-responsive. The diagnosis of homocystinuria caused by CBS-- in patients was made at a mean age of 24.7years (4-54), by establishing sever hyperhomocysteinemia and homocystinuria, hypermethioninemia, and decreased level of cysteine in plasma. Furthermore, CBS activities measured in extracts of cultured fibroblasts were less than 2% of the mean in controls, except in 1 patients, in whom we observed CBS activites in the heterozogous range. However in this patient we were able to show a defective CBS regulation by S-adenosylmethionine (AdoMet) leading to severe hyperhomocysteinemia. Up to now, in 18 of 24 patients homozygous CBS deficiency was confirmed by molecular genetic analysis of the CBS cDNA…. Deep venous thrombosis was diagnosed by means of flebography and pulmonary embolism by perfusion-ventilation scintigraphy….. Results. Of 24 CBS-- patients, 3 individuals, all belonging to the same kindred, were carriers of FVL; no homozygotes for FVL were observed. In the study group, 6 individuals, mean age 23years (9-40y) suffered from a thrombotic complication; 4 patients had deep venous thrombosis and 2 patients had pulmonary embolism…. Venous thrombosis occurred in these 6 patients before the start of homocysteine lowering treatment, which had been prescribed immediately after the diagnosis of homocystinuria had been established. All 6 patients with thrombosis proved to be VitB6-reponders." (Continues)	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
(Continued) Kluijtmans LAJ et al (1998); Blood; U Hospital Nijmegen, Nijmegen, The Netherlands	(Contin) 24 CBS-- Dutch cases, 23/24 VitB6-responsiv, Possibly not all of Dutch CBS-- cases known at the time n = 5 n = 16	(Continued) Note: Using the data from Mudd et al (1985), there would have been expected to have been approx 10 thromboembolic events in the period preceeding diagnosis, and in fact there were only 6 such, which raises the question of whether these Dutch VitB6-responsive mutations might be less severe than those of Mudd et al, or alternatively whether some other Dutch factor (environment/genetic) is more protective than that of Mudd et al, but the difference would probably not be ss on the basis of low n alone, but might go close…… Unfortunately the (then) present age of the group was not provided, so the analogous comparison for the treated period is not possible. (See Kluijtmans et al (1999) regarding this) Results. There was 1 venous thrombotic event amongst the 3 FVL heterozygotes. There were 5 venous thrombotic events amongst the 21 FVL wild-type homozygotes. So, some difference (33% vs 24%) but certainly not ss on the basis of low n alone. There was 3 venous thrombotic events amongst the 5 C677T TT homozygotes. There was 1 venous thrombotic event amongst the 11 C677T CT heterozygotes. There were 2 venous thrombotic events amongst the 8 C677T CC wild-types. So, some difference (60% vs 9% vs 25%) (or 60% vs CT + CC 16%) but would certainly not be ss for the trichotomous comparison trendwise, while possibly would be ss for the dichotomous comparison (oddly enough, neither of these were tested for ss, while tests for the difference in genotypes of CBS—vs the Dutch general population were….?.....) "The total amount of nonprotein-bound homocysteine was calculated as twice the concentration of homocystine plus the concentration of the Hcy-Cys mixed disulfide" C677T Methionine fHcy TT 75uM 155uM CT + CC 117uM 128uM P = 0.4 P = 0.6 In lieu of treatment details not provided by the authors here, I provide the following from their (1999) ( ) work summarised further below: "Pyridoxine responsiveness was examined after 6 weeks of treatment with VitB6 750mg/day in adults or 200-500mg/day in children. Patients in whom non-protein-bound serum Hcy had decreased to <20uM, or tHcy to <50uM, were classified as pyridoxine responsive. All other patients were categorized as pyridoxine-nonresponsive homocystinurics……Long-term Hcy-lowering therapy (mean term, 13years (1-29y) consisted of maximally 75mg pyridoxine. 15 (56%) patients were concomitantly treated with 5mg/day folic acid, and 8 (30%) patients also with betaine 6mg/day. Only 1 patient had a methionine-restricted diet, with a methionine content of 600 mg/day. Intramuscular injections with 1mg hydroxycobalamin every 1-2months were given to 4 patients, because of development of a VitB12 deficiency." (Note: This info for a group of 29 CBS--, which very likely included the very large majority of the 24 comprising their study summarised here) .	tHcy >195uM >168uM	VitB6 <=75mg/d +/- Folate 5mg/day +/- Betaine 6mg/day +/- VitB12 1mg/1-2months, And, 1 patient on low-Met diet. (see column 2 to left for further n details.

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Megnien J-L et al (1998); Circulation; Broussais & Necker Hospitals, Paris, France.	14 (10 CBS--, + 4 ???- -) 15 Their controls 15 CBS-+ obligate heterozyg parents 15 Their controls	"14 subjects with homozygous homocystinuria (10 CBS-- and 4 defects of remethylation), including 2 pairs of siblings (I think a comma should be here. DV) aged 3-34years (mean 13years) and 15 of their heterozygous parents coming from 8 families and aged 32-47y (mean 41y) underwent ultrasonographic investigation of the carotid artery between 1996 and 1997. They were compared with 2 control groups of healthy subjects of similar ages studied with the same technique of carotid investigation during the same period…..The age at diagnosis of homozygous subjects ranged from 0-22years (mean 5years)." "At the time of investigation, 12 homozygous subjects were consuming a low methionine diet, 3 were taking pyridoxine (of whom 2 were pyridoxine sensitive), 6 were taking folic acid supplements, 3 were taking cobalamin, and 11 were prescribed betaine. 3 homozygous subjects had a previous (Assume pre-diagnosis?..DV) history of cardiovascular disease, including 1 cerebral trunk thrombosis, 1 stroke with iliac artery stenosis, and 1 cerebral arterial spasm with iliac artery thrombosis. (Note: Assuming the 8 CBS-- had a mean age of diagnosis of 5y, then according to the data of Mudd et al (1985) 0.3 thromboembolic events would have been expected to have occurred prior to diagnosis/treatment – the shortcomings of attempting any such comparisons given the data incompleteness is fairly obvious. DV). No homozygous subject had any ultrasonic evidence of arterial narrowing or plaque in the carotid arteries. Heterozygous subjects had no present or past history or sign of cardiovascular disease and did not take any cardiovascular drug treatment, but 1 subject had ultrasonic evidence of plaque in the left carotid artery bifurcation." "Arterial Investigations. A high-resolution ATL Ultramark 9 B-mode ultrasound system was used to measure IMT in the far wall of the right common carotid artery….Simultaneously, the lumen diameter was imaged between the far-wall and near-wall lumen-intima interfaces (leading edges), frozen in telediastole, and transferred to the computer for automated measurement with the edge-detection program. The cross-sectional area of the intima-media complex (CSA-IMC) was calculated from IMT and lumen diameter as pye*IMT*(IMT+D)."		

	Age	Smoking	HT	Diab	HypChol	IMT	Diam	CSA-IMC	tHcy	
14 (10 CBS--, + 4 ???- -)	13y	1.4py	0	0	2	0.50mm	5.00mm	8.69mm^2	91uM (9-179)	Varied, see column two to right above - note at least 2 of the remethylation defects must have been on a low-Met diet….. inappropr
15 Their controls						0.44mm P<.001	5.31mm P<.05	7.81mm^2 P<.05		
15 CBS-+ obligate heterozyg parents	41y P<.001	7.5py P<.05	3 nss	0 nss	4 nss	0.50mm	5.57mm	9.57mm^2	15uM (4-56) P<.001	
15 Their controls						0.48mm nss	5.93mm nss	9.65mm^2 nss		

Where: py = pack years, HT = HyperTension, Diab = Diabetes,
IMT = Intima-Media Thickness, Diam = diameter,
CSA-IMC = the Cross-Sectional Area of the Intima-Media Complex,
The latter three measures being those adjusted for body surface area, chosen as the most meaningful, and not in much disagreements with the other versions provided.

(Continues)

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Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
(Continues) Megnien J-L et al (1998); Circulation; Broussais & Necker Hospitals, Paris, France.	(Contin) 14 (10 CBS--, + 4 ???- -) 15 Their controls 15 CBS-+ obligate heterozyg parents 15 Their controls	(Continues) Unfortunately, it is not possible from the data/figures provided to determine whether the Diam and CSA-IMC measures of the four remethylation defects are likely to differ from those of their controls, while it is possible to say that the average of their IMT must have been higher than all but 1 of their controls, and this might have been ss. In two multiple regression models with Age being the only other ss (P = .04, .02 respectively) IV in the model(s), body surface area and height (respectively) being nss, tHcy was ss (P = .008, .008 respectively), and R2 was 0.25, 0.27 (respectively), in predicting IMT. Something similar applied for predicting CSA-IMC, though the lower ss do not seem to be in accord with the higher R2 values?.... tHcy was not similarly a ss (was vnss) predictor of Diam.		
De Stefano V et al (1998) ; Ann Hum Genet; 11 European Countries	785 individ's particip'g in the European Athero-sclerosis Research Study II (EARSII), from 11 countries across Europe	"Summary. We have examined 4 apparently non-functiional polymorphisms in the CBS gene and have determined their frequency, degree of linkage disequilibrium and association with plasma Hcy levels. The polymorphisms are a 68bp insertion in exon 8, C699T in exon 8, C1080T in exon 11 and C1985T in the 3' untranslated region. 785 individuals participating in the European Atherosclerosis Research Study II (EARSII), from 11 countries across Europe were genotyped for these polymorphisms. The 68bp insertion ahd the highest frequency in the UK and in the middle region, with a lower frequency in the Baltic and the South (P = .01), and the exon 11 polymorphism had the highest frequencies of the rare allele in the Baltic (P<.05). There was a high degree of linkage disequilibrium between the polymorphisms (P<.001 overall), except between C699T and the C1985T, with 3 common haplotypes accounting for nearly 80% of the chromosomes. Examination of the association between these polymorphisms and plasma Hcy levels revealed that the carriers of the rare alleles of the C699T, C1080T and C1985T polymorphisms had lower plasma Hcy concentrations than those homozygous for the common alleles, although these differences were not ss. The thermolabile valine variant caused by a substitution of a C for a T at nucleotide 677 in the methylenetetrahydrofolate reductase (MTHFR) has previously been shown to have profound effects on plasma levels of Hcy in this sample, but the Hcy-raising effect associated with this thermolabile variant was not seen incarriers of the 68bp insertion, with this interaction being ss (P<.001). These data demonstrate that variation in the CBS gene as detected with these 4 polymorphisms, had no ss effect on plasma Hcy levels in these healthy young men. However, the presence of the 68bp insertion, which is found in approximately 7.5% of individuals in the population of Europe sampled, abolishes the raising effect of thermolabile MTHFR Val/Val genotype…."		

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Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Kluitjmans LAJ et al (1999); Am J Hum Genet; U Hospital Nijmegen, Nijmegen, The Netherlands	29(27) CBS--, Dutch, Very roughly an even mixture of VitB6-responsiv and non-responsiv, no newborn screening done	"Patients. We studied 29 patients with homocystinuria due to CBS--, from 21 unrelated pedigrees. Patients were initially diagnosed on the basis of clinical manifestations of CBS--, in combination with a quantitative determination of severe hypermethioninemia. In 2 patients (19, 29), the diagnosis was made only on the basis of severe homocystinuria, which was demonstrated by qualitative urine analyses. Homozygous CBS-- in these 2 patients has been confirmed by detection of a homozygous mutation (patient 19) and by the clinical manifestation of ectopia lentis (patient 29), never observed in CBS-+…. Pyridoxine Responsiveness. Pyridoxine responsiveness was examined after 6 weeks of treatment with VitB6 750mg/day in adults or 200-500mg/day in children. Patients in whom non-protein-bound serum Hcy had decreased to <20uM, or tHcy to <50uM, were classified as pyridoxine responsive. All other patients were categorized as pyridoxine-nonresponsive homocystinurics…… 14 (48%) of 29 patients were classified as pyridoxine responsive and 9 (31%) patients as pyridoxine nonresponsive….. In 6 patients (12, 13, 16, 21, 28, and 29), responsiveness to pyridoxine alone could not be assessed. In view of their extremely high homocysteine levels at diagnosis, these patients were treated directly with a combination of therapeutic regimens (pyridoxine and folic acid, with or without betaine). Pyridoxine responders were diagnosed at a mean age of 29years (median 26y, range 7-45y), and nonresponders at a mean age of 19years (median 16y, range 4-30y); P = .08. 7 (58%) of 12 homozygotes for the I278T mutation showed in vivo pyridoxine responsiveness, 3 (25%) were nonresponders, and, in 2 (17%) patients, this specific responsiveness could not be assessed. In 17 individuals with other genotype combinations, including compound heterozygotes for I278T, these numbers were 7 (41%), 6 (35%), and 4 (24%), respectively. Conversely, in 14 pyridoxine-responsive patients 18 (64%) of 28 alleles carried the I278T mutation (7 homozygotes, 4 heterozygotes), versus 6 (33%) I278T alleles in 9 nonresponsive patients (3 homozygotes). There was an absolute concordance of pyridoxine responsiveness between siblings. Response to Homocysteine-Lowering Treatment. Long-term Hcy-lowering therapy (mean term, 13years (1-29y) consisted of maximally 75mg pyridoxine. 15 (56%) patients were concomitantly treated with 5mg/day folic acid, and 8 (30%) patients also with betaine 6mg/day. Only 1 patient had a methionine-restricted diet, with a methionine content of 600 mg/day. Intramuscular injections with 1mg hydroxycobalamin every 1-2months were given to 4 patients, because of development of a VitB12 deficiency. 2 patients could not be followed after diagnosis had been made: patient 6 refused treatment and patient 13 moved to another country. Hence follow-up was recorded in 27 (93%) of the patients with homocystinuria. The mean length of follow-up was 11y (range 1-20y, n = 11) in homozygotes for the I278T mutation and 16years (range 3-27y, n = 16) in patients with other genotypes. Biochemically, pyridoxine treatment resulted in a marked decrease in Hcy concentrations of 90% in homozygotes for the I278T mutation and of 67% in patients with other genotypes (P<.02). Extended intervention with folic acid, with or without betaine, further decreased Hcy concentrations in both genotype groups by 84% and 47% respectively (P<.05). Hcy concentrations normalized (ie fHcy<20uM or tHcy<50uM) in 21 (78%) of 27 patients. ……."		
(Continued				

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
(Continues) Kluitjmans LAJ et al (1999); Am J Hum Genet; U Hospital Nijmegen, Nijmegen, The Netherlands	(Contin) 29(27) CBS--, Dutch, Very roughly an even mixture of VitB6-responsiv and non-responsiv, no newborn screening done	(Continues) fHcy 11 I278T homozygotes 13uM 16 other genotypes 23uM P = 0.3 "On the basis of genotype classification, 121 patient-years of treatment were recorded in 11 homozygotes for the I278T mutation, during which, in 1 patient (7)(VitB6-responsive. DV), peripheral arteriosclerosis, already present at diagnosis, proceeded to development of an abdominal aortic aneurysm that required vascular surgery. During 257 patient years of treatment in patients with another genotype, 1 patient (25)(VitB6-responsive) who suffered from a cerebrovascular event before diagnosis died, at age 42y, of a myocardial infarction. In summary, in 378 patient-years of Hcy-lowering treatment, only 2 vascular events were recorded. From the data of Mudd et al (1985), an expected number of primary vascular events of 2 per 25years can be calculated for untreated patients. Without treatment in our group fo 27 homocystimurics, at least 30 vascualr events would have been expected (P<.01). During long-term follow-up, no first involvement of any involved organ system was noticed. Furthermore, no deterioration fo clinical symptoms was recorded, except for recurrent depressive episodes in 2 patients (2, 15)." Now, as the data provided does not enable the outcomes in terms of thromboembolic events to be related to patient-years of treatment with regard to VitB6-responsiveness, the only appropriate comparison with regard to Mudd et al's (1985) data would be that of the combined whole-group here with that of Mudd et al. So Roughly averaging the age at diagnosis for the whole group is by [14*29years + 9*19years]/(14 + 9 = 23) = 25years, this being for the 14 known VitB6-responsives with the 9 known VitB6-nonresponsives, and assuming representativity for the other 6 with undetermined VitB6-responsiveness. And, 378 patient-years of treatment for the whole group of 27 cases, so the average age 'now' is by 25years + 378py/27p = 39years. Now, referring these ages to Mudd et al's (1985) Time-to-event graph for first thromboembolic event, the proper method is to subtract the probability of having had a thromboembolic event by age 39y (P = 0.59) from that by age 25y (P = 0.43), that is P = 0.19, then to multiply this by the number of patients, 27, such that 0.19*27 = 4.3 (4 and 3tenths) thromboembolic events would have been expected had the group been untreated, during the period of treatment. This is obviously in great disagreement with their claim here of 30 thromboembolic events being expected given no treatment, and far from ss being P<.01 as claimed, in fact the difference would be nss. Furthermore, as analogously noted in my analysis of their earlier (1998) work, using the data from Mudd et al (1985), there would have been expected to have been approx 0.43*27 = 11.6 first thromboembolic events in the period preceeding diagnosis, and in fact there were only 8 such, which raises the question of whether these Dutch CBS-- might be less severe than those of Mudd et al, or alternatively whether some other Dutch factor (environment/genetic) is more protective than that of Mudd et al, but the difference would probably not be ss, on the basis of low n alone – in any case the ramification for adjustment of their thromboembolic ss to less ss. Of the 8 cases presenting with a thromboembolic event, only 2 (25%) also had arteriosclerosis on presentation, and there were 7 (24%) presentations with arteriosclerosis out of the 29 in the whole group.	tHcy 30uM 67uM P<.01	See column 2 to left, above

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Peter-schmitt MJ et al (1999); NEJM; New England Newborn Screening Program, Boston, USA	USA newborn screening	"For the first 23.5years of the review period, the blood methionine cutoff value was 2mg/dl (134uM). Among the 2.2million infants screened during that period, 8 with homocystinuria were identified (1:275,000). In 1990, the cutoff value was reduced to 1mg/dl (67uM). Among the 1.1million infants screened in the subsequent 8.5years, 7 with the disorder were identified (1:157,000). During the latter period, the specimens were collected from 6 of the 7 infants when they were 2days of age or less; 5 of the 6 had blood methionine concentrations below 2mg/dl. Use of the reduced cutoff level increased the false positive rate from .006% (16.5: 275,000) (1:16,700) to .03%. (47.1:157,000) (1:3,330)."		
Yap S et al (1999); Thromb Haemost; The Children's Hospital, Dublin, Ireland	(Assume from their 1998 work see above) All 26 CBS-- detected in Ireland 1971-97/98?. 12 females, 19 families. 25/26 VitB6-non-responsiv	"Activated Protein C resistance is currently the most common hereditary cause of thrombophilia, accounting for 21% of deep vein thrombosis in individuals under 70years of age and for up to 50% of familial venous thrombosis (cites Rees et al 1995). A missense mutation in coagulation factor V (Factor V Leiden, FVL) accounts for 95% of cases with activated Protein C resistance (cites Bertina et al 1994 and Dahlback 1995). This single point mutation in the gene coding for Factor V (G1691A) predicts the synthesis of the FVL molecule, where glutamine has been substituted for arginine at position 506 (Arg506Gln). This renders the molecule resistant to inactivation by activated Protein C which results in a hypercoagulable state conferring a lifelong increased risk of thrombosis. The allelic frequency of FVL in Europe is about 4.4% with the highest frequency found in Greeks at 7%. The estimated risk of thrombosis in conjunction with FVL is 5-10 fold in heterozygotes and 50-100 fold in homozygotes." "…26 individuals with homocystinuria (median age 17.6years, range 3.5-32.8years) and 36 obligate heterozygotes (median age 51.5years, range 34-74years) were screened for FVL. All the homocystinuric individuals received treatment, except one, within 6weeks of birth for those who were diagnosed at birth through the national newborn screening programme (n = 20) and at the time of diagnosis in those late detected (n = 5, mean age of atarting treatment 4.9years, range 1.4-11years). All had been free from venous thrombosis, except one CBS-- and one CBS-+. Neither of the 2 individuals with venous thrombosis carried FVL. 2 independent CBS-- (ages 15, 18y) were heterozygous for FVL (allelic frequency 3.8%) and 3 independent CBS-+ (ages 40, 46, 46y) were also heterozygous for for FVL (allelic frequency 4.2%)." (Where tHcy was derived using the relationship of fHcy(ine) (data values provided by Yap and Naughten here) to tHcy reported by Bonham et al (1997), such that: tHcy = 60 + 4.5(fHcy(ine)) for values of fHcy(ine) < 20uM, and tHcy = 60 + 90 + (fHcy(ine) – 20) for values of tHcy(ine) >=21uM	tHcy = 107uM, range 80 – 180uM	See Yap et al 1998 above

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Yap S et al (2000); Sem Thromb Hemost; Meta-analysis Irish, Australian & Dutch	84 CBS--,	"Abstract……The study on the natural history of untreated CBS-- disclosed that, at the time of maximal risk, in other words beyond 10years old, there was one vascular event (VE) per 25years. Recent studies from Australia (n = 32), The Netherlands (n = 28), and Ireland (n = 24) have documented the effects of long-term treatment on the vascular outcome of a total of 84 patients with 1314 patient-years of treatment for CBS--. The mean (range) age was 27.8 (2.5 to 70) years. 5 VE were recorded during treatment; 1 pulmonary embolism, 2 myocardial infarctions, and 2 abdominal aneurysms. All 5 VE occurred in VitB6-responsive patients at a mean (range) age of 48.8 (30-60) years. In 1314patient-years of treatment, 53 VE would have been expected if they remained untreated; instead only 5 were documented, relative risk = .091 (95%CI .043 – 0.190)." P <.001 Now, firstly, it was not proper to aggregate thromboembolic events in with aneurysms, as they may well have differing etiologies, and also as diagnosis is not assured. Secondly, the method of calculation of the number of events expected in the treatment period had treatment not been applied is by no means made clear. Taking the average age of 27.8years, minus the total treatment time divided by n (1314/84 = 15.6years) one derives that on average treatment commenced at age 27.8y – 15.6y = 12.2y. Going to the thromboembolic event time-curve of Mudd et al (1985) it can be seen that for an average individual of this group, the probability of having a thromboembolic event was 0.47 (by age 27.8y) – 0.12 (by age 12.2y) = 0.35. And, 0.35*84 = 29.4 thromboembolic events expected during the treatment period had treatment not been applied. By coincidence the risk ratio would be somewhat similar to the one given. More to the point however, is that the treatments applied by these three different groups of practitioners are quite different, in particular the Irish compared to the other two, and analysis would more profitably had compared on this basis, and sought biochemical theorietical basis for the differences amongst them, rather than proceeding as they have done. See also Yap & Naughten (1998), Kluitjmans et al (1999), Wilcken & Wilcken (1997) all above, as well as for comparison Walter et al (1998) above. See also my analysis of these noted aspects below.	See respective studies as noted	See respective studies as noted
Tangerman A et al (2000); Metabolism Various sources of patients, possibly The Netherlands, Australia, USA, & elsewhere	22 CBS--, selected to have higher meth-ionine levels	"To assess the ability of patients with CBS-- to perform the reactions of the methionine transamination pathway, the concentrations of the products of this pathway ("Transamination metabolite values are expressed as the sum of methanethiol released sequentially into the gas phase at pH 7 (protein-S-S-CH3), pH 10 (X-S-S-CH3), and pH 12.5 to 13 (chiefly 4-methylthio-2-oxo-butyrate)".) were measured in plasma and urine. The results clearly demonstrate that CBS-- patients develop elevations of these metabolites once a threshold of 350uM for the concurrent plasma methionine concentration is exceeded. The absence of elevated methionine transamination products previously reported amongst 16 CBS—VitB6-responsive patients may now be attributed to the fact that in those patients the plasma methionine concentrations were below this threshold. The observed elevations of transamination products were similar to those observed among patients with isolated hypermethioninemia. Plasma Hcy did not exert a consistent effect on thransamination metabolites, and betaine appeared to effect transamination chiefly by its tendency to elevate methionine." To describe the scatterplots, firstly the results for those on betaine treatment not have been differentiable from those not. For plasma the upper level of the reference range is given as 0.5uM – once the plasma methionine levels come above approx 350uM the transamination products are fairly widely scattered but increasing with the methionine – up to methionine 600uM, mostly 1 to 2uM – from methionine 600 – 15000uM, plenty of values all the way from 2 to 20uM. For urine the scatterplot is entirely in agreement with that for the plasma, in its proportions with regard to the reference range. It seems unlikely that sulfate production dimished in CBS-- is fully restored by methionine metabolism through transamination.		

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Lentz SR et al (2000); Am J Physiol Heart Circ Physiol; U Iowa, Iowa, USA	CBS++ & CBS-+ littermate mice.	"We have used genetic and dietary approaches to produce hyperhomocystinemia in mice. CBS-+ mice, which have a selective defect in Hcy transsulfuration, and wild-type CBS++ littermates were fed either a control diet or a diet that is relatively deficient in folic acid for 6weeks. Plasma tHcy was 5.3+/-0.7uM in CBS++ mice and 6.4+/-0.6uM in CBS-+ mice (P = 0.3) given the control diet. Plasma tHcy was 11.6+/-4.5uM in CBS++ mice and 25.1+/-3.2uM in CBS-+ mice (P = .004) given a low-folate diet. In mice fed the control diet, relaxation of aortic rings in response to the endothelium-dependent vasodilator acetylcholine did not differ significantly between CBS++ and CBS-+ mice. In contrast, in mice fed a low-folate diet, maximal relaxation to acetylcholine was markedly impaired in CBS-+ mice (58+/-9%) comparted with CBS++ mice (84+/-4%) (P = .01). No differences in relaxation to the endothelium-independent vasodilator sodium nitroprusside were observed among the four groups of mice."
Weiss N et al (2001); Arterioscler Thromb Vasc Biol; Boston U School of Medicine, Boston, USA.	CBS++ & CBS-+ littermate mice.	"Previous in vitro experiments have shown that hyperhomocystinemia leads to oxidative inactivation of nitric oxide, in part by inhibiting the expression of cellular glutathione peroxidase (GPx-1). To elucidate the role of intracellular redox status on Hcy-induced endothelial dysfunction and oxidant stress, CBS-+ and wildtype CBS++ mice (fed standard chow (LabDiet 5001, PMI Nutrition International) ad libitum. DV) were treated with the cysteine donor L-2-oxothiazolidine-4-carboxylic acid (OTC). CBS-+ mice had significantly lower GPx-1 activity compared with their CBS++ littermates, and OTC treatment led to a modest increase in tissue tissue GPx-1 activity and significant increases in total thiols and in reduced glutathione levels in both CBS++ and CBS-+ mice (OK, see below. DV). Superfusion of the mesentery with Beta-methacholine or bradykinin produced dose-related vasodilation of mesenteric arterioles in CBS++ mice and and in CBS++ mice treated with OTC. In contrast, mesenteric arterioles from the CBS-+ mice manifested dose-dependent vasoconstriction in response to both agonists. OTC treatment of CBS-+ mice restored normal microvascular vasodilator reactivity to Beta-methacholine and bradykinin. (OK. DV) These findings demonstrate that mild hyperhomocysteinemia leads to endothelial dysfunction in association with decreased bioavailable nitric oxide. Increasing the cellular thiol and reduced glutathione pools and increasing GPx-1 activity restores endothelial function." "The levels of total thiols and of glutathione were 15- to 20-fold higher in hepatic tissue compared with cardiac tissue. In cardiac tissue, the levels of total thiols and of total, reduced, and oxidised glutathione, as well as the ratio of reduced to oxidized glutathione increased to a more reduced state (with OTC treatment, ss for CBS-+, but (& some ss) also very much in agreement for CBS++. DV) (& note total thiols 3.79 vs 3.16 respectively with OTC, but otherwise no difference anywhere. DV). The total thiols and glutathione levels were more variable in hepatic tissue and did not differ significantly between CBS++ and CBS-+ mice nor between untreated and OTC-treated mice. (overall CBS-+ > CBS++, unexpected; CBS-+(-OTC) > CBS-+(+OTC), unexpected, except for total thiols which were exactly equal, unexpected (one wonders therefore if the data columns hadn't been switched by mistake.); CBS++(-OTC) < CBS++(+OTC), as expected. DV" Note: taken overall, it is fair to say that the physiological changes documented bear no relationship to the levels of thiols and glutathione documented, unless the cardiac tissue is more representative of the vascular tissue tested, than is the liver. In this and in any case the liver thiol situation here is completely enigmatic…. "As shown previously in 20week old CBS-+ mice (cites Weiss et al 2000), 10-12week old CBS-+ mice had significantly lower hepatic cellular GPx-1 activity compared with their CBS++ littermates (620 vs 540, ss). 1week of treatment with OTC led to a 20% and 30% increase in cellular GPx-1 activity in both CBS++ and CBS-+ mice, respectively. After OTC treatment, the enzyme activity was not different between CBS++ and CBS-+ mice (740 vs 700, nss)" "Plasma levels of soluble P-selectin were 40% higher in CBS-+ mice compared with CBS++ mice. 1week of treatment with OTC normailized plasma P-selectin concentrations to values not different from those in CBS++ mice. OTC treatment had no effect on plasma P-selectin values in CBS++ mice (ELISA method DV). Immunostaining of aortic sections of mice with anti-P-selectin antibody showed more intense staining of the luminal surface of the endothelialcell layer in CBS-+ mice compared with CBS++ mice, consistent with increased endothelial P-selectin expression. In some sections, platelet-rich thrombi were seen adherent to the endothelial surface in CBS-+ mice but not in CBS++ mice. OTC treatment suppressed the increase in P-selectin expression in aortic sections of CBS-+ mice to levels observed in untreated CBS++ mice. (Purportedly representative section photographs support this. DV)" Note: No formal tests of statistical significance of these latter were reported.

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Davi G et al (2001); Circulation; U Chieti, U Naples Federico II, Italy.	13 CBS--, 12 VitB6-responsiv, 1 nonrespon	"…Hcy may exert its effects by promoting oxidative damage. In the present study, we investigated whether in vivo formation of 8-iso-ProstaglandinF2alpha (8-iso-PGF2alpha), a platelet-active product of arachidonic acid peroxidation, is enhanced in CBS-- and whether it correlates with in vivo platelet activation, as reflected by thromboxane (TX) metabolite excretion…. Urine and blood samples were obtained from patients with CBS-- (n = 13, 7 male, age 14-51y, belonging to 8 unrelated families) and age-matched healthy subjects. Urinary 8-iso-PGF2alpha excretion was significantly higher in CBS—than in control subjects (640+/-384 vs 213+/-43pg/mg creatinine; P = .0015) and correlated with plasma Hcy (rho = 0.398, P = .0076). Similarly, urinary 11-dehydro-TXB2 excretion was enhanced in CBS—(1166+/-415 vs 324+/-72pg/mg creatinine; P = .0015) and correlated with urinary 8-iso-PGF2alpha (rho = 0.362, P = .0153). VitE supplementation (600mg/day for 2weeks) was associated with a ss increase in its plasma levels (from 16.6+/-4.6 to 40.4+/-8.7uM, P = .0002) and with reductions in 8-iso-PGF2alpha (from 790+/-159 to 559+/-111pg/mg creatinine, P = .018) and 11-dehydro-TXB2 (from 1273+/-383 to 913+/-336pg/mg creatinine, P = .028). A ss inverse correlation was found between urinary 8-iso-PGF2alpha and plasma VitE levels (rho = -0.745, P = .0135), although 2weeks of VitE supplementation at 600mg/day failed to normalize enhanced lipid peroxidation " "….ectopia lentis, osteoporosis, and different degrees of mental retardation. All but 1 were responsive to pyridoxine and had been on pyridoxine treatment (600-900mg/day) from the time of diagnosis. The pyridoxine-unresponsive patient was on betaine 8g/day." Average age at diagnosis was 15.3years, and average duration of treatment was 16.9years, therefore average age now (then) is 32.2years. Using the time-to-event curve for first thromboembolic event curve from Mudd et al (1985) the number of thromboembolic events expected during the treatment period had treatment not been used was (0.5 – 0.13 = 0.37) * 13 = 4.8. "None of the CBS-- patients showed ST depression or Q waves on the ECG, and their clinical records were negative for angina pectoris, myocardial infarction, and venous thromboembolism. All had normal peripheral pulses and were negative for bruits over the caratid vessels. Duplex scanner analysis confirmed the negativity with respect to carotid arteries, whereas it revealed early signs of iliac stenosis (n = 1), ankle/arm ratio <0.85 (n = 3), and 1 of these also exhibited a chronic vein insufficiency." The difference between the observed 0 thromboembolic events during treatment and the 4.8 expected during that treatment period if treatment had not been applied is almost ss at the .05 level.	CBS-- tHcy = 113uM, range 17-287uM	VitB6 600-900mg/d only(?) for responsiv, betaine 8g/day for the 1 VitB6-nonrespon
Trondle U et al (2001) ; Acta Med Austriaca ; U Vienna, Vienna, Austria.	2 CBS-- VitB6-semi/non-responsiv	"…We identified the CBS 833T>C/1058C>T and CBS 828ins104/1358del134 comp hetz genotype in our index patients. Both patients showed mental retardation and ectopia lentis. CBS 833T>C/1058C>T was associated with severe vascular complications (1week post-surgery for ectopia lentis at age 25years, approx time of CBS--diagnosis with tHcy approx 190uM, Cysteine 1.7uM (ref 30-80uM)), which was not the case for CBS 828ins104/1358del134 (neonatal diagn, no treatment till age 29y?..). The patient with CBS 828ins104/1358del134 was negative for FVLeiden, F2G20210A, MTHFR C677T, and MTHFR A1298C, while the patient with CBS 833T>C/1058C>T was heterozygous for MTHFR A1298C. A combination therapy including pyridoxine, folic acid, hydroxycobalamin, and betaine failed to lower tHcy plasma levels below 50uM in both patients."	tHcy 145, 420uM on VitB6 alone, respect'ly	No treatment till ages 25, 29years?...

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Yap S et al (2001a); Arterioscl Thromb Vasc Biol; Meta-analysis Irish, Australian Dutch and UK.	158 CBS--	"Abstract…..We performed a multicenter (Dublin, Ireland; Manchester, UK, London, UK, Nijmegen, The Netherlands; Sydney, Australia. DV) observational study to assess the effectiveness of long-term Hcy-lowering treatment in reducing vascular risk in 158 patients. Vascular outcomes were analysed and effectiveness of treatment in reducing vascular risk was evaluated by comparison of actual to predicted number of vascular events, with the use of historical controls from a landmark study (Mudd et al 1985. DV) of 629 untreated patients with CBS--. The 158 patients had a mean (range) age of 29.4 (4.5 to 70) years; 57 (36%) were more than 30years old, and 10 (6%) were older than 50years. There were 2822 patient-years of treatment, with an average of 17.9years per patient. Plasma Hcy levels were markedly reduced from pre-treatment levels but usually remained moderately elevated. There were 17 vascular events in 12 patients at a mean (range) age of 42.5 (18 to 67) years: pulmonary embolism (n = 3), myocardial infarction (n = 2), deep venous thrombosis (n = 5), cerebrovascular accident (n = 3), transient ischemic attack (n = 1), sagittal sinus thrombosis (n = 1), and abdominal aortic aneurysm (n = 2). Without treatment, 112 vascular events would have been expected, for a relative risk of .09 (95%CI .036 to 0.228; P<.0001)." Now, firstly, it was not proper to aggregate thromboembolic events in with aneurysms, as they may well have differing etiologies, and also as diagnosis is not assured. Secondly, the method of calculation of the number of events expected in the treatment period had treatment not been applied is by no means made clear. Taking the average age of 29.4years, minus the total treatment time divided by n (2822/158 = 17.9years) one derives that on average treatment commenced at age 29.4y – 17.9y = 11.5y. Going to the thromboembolic event time-curve of Mudd et al (1985) it can be seen that for an average individual of this group, the probability of having a thromboembolic event was 0.52 (by age 29.4y) – 0.10 (by age 11.5y) = 0.42. And, 0.42*158 = 66.4 thromboembolic events expected during the treatment period had treatment not been applied. Taking away the 2 abdominal aortic aneurysms to leave 15 vascular events instead of the 17 noted, the relative risk would in fact be closer to 0.2 than the .09 claimed. More to the point however, is that the treatments applied by these different groups of practitioners are quite different, in particular the Irish compared to the Dutch and the Australian (see Yap et al 2000 above) other two, and, one needs to know the amino acid supplementation used by the two UK groups – given their average age of treatment commencement it is probable that they do not achieve (if they even attempt it…) compliance in this respect the equal of the Irish group. Analysis would more profitably had compared on this basis and sought biochemical theorietical basis for the differences amongst them, rather than proceeding as they have done. See also Yap & Naughten (1998), Kluitjmans et al (1999), Wilcken & Wilcken (1997), Yap et al (2000) all above, as well as for comparison Walter et al (1998) above. See also my analysis of these noted aspects below. Dublin Sydney Nijmegen Manchester London n CBS-- 28 40 30 31 41 n CBS-- followed 27 32 28 30 41 n VitB6-responsive followed 1 17 19 8 25 Total VitB6-resp treat-years 13.7 315 250 183.1 482 AverageVitB6-resp treat-years 13.7 18.5 13.2 22.9 19.5 n VitB6-nonrespons followed 26 15 9 22 16 Total VitB6-nonr treat-years 444.0 288 169 385.5 291 Average VitB6-nonr treat-years 17.1 19.2 18.8 17.5 18.2 n thrombo events 0 2 1 3 9in4pts Diet Met/day restriction 200-625+aaCys GenAdvice 600 160-900 400-1375 VitB6 100-800mg/d 100-200mg/d 200-750mg/d 50-500mg/d 20-500mg/d Folate 5mg/day 5mg/day 5mg/day 5mg/day 5-10mg/d VitB12 if deficient to all if deficient nil 50ugoral Betaine 3-6g/day 6-9g/day 6g/day 4.5-15g/day 2-6g/day Note: No further detail supplied differentiative of VitB6-responders from –nonresponders…. Venupuncture >=8-10/year 1-4/year 1-2/year 1-4/year 2-4/year Amino Acid Suppl'n ???

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Yap S et al (2001b); J Inher Metab Dis; The Children's Hospital, Dublin, Ireland	23 CBS—VitB6-nonresponsives, (13 early-detected good-compliance, 6 early-detected poor-compliance, 2 detected at 2years good-compliance, 2 untreated at 22, 12years, & 10 sibling controls	"Summary:….The mental capabilities of 23 pyridoxine-nonresponsive individuals with 339 patient-years of treatment were assessed (by 1 blinded psychologist. DV) using age-appropriate psychometric tests and were compared to those of 10 unaffected siblings (controls). Of the 23 individuals, 19 were diagnosed through newborn screening with early treatment, 2 were late-detected and 2 were untreated at the time of assessment. 13 of the newborn, screened group who were compliant with treatment had no complications, while the remaining 6, who had poor compliance, developed complications. Good compliance was defined by a lifetime plasma free homocystine median <11uM (equivalent to tHcy < 110uM. DV). The newborn screened, good compliance group (n = 13) with a mean age of 14.4years (range 4.4-24.9) had a mean full-scale IQ (FIQ) of 105.8 (range 84-120), while the poorly-compliant group (n = 6) with a mean age of 19.9years (range 13.8-25.5) had a mean FIQ of 80.8 (range 40-103). The control group (n = 10) with mean age of 19.4years (range 9.7-32.9) had a mean FIQ of 102 (range 76-116). The 2 late-detected (at ages 2.4, 2.9years) patients aged 22.4 and 11.7years had FIQ of 80 and 102, while the 2 untreated patients aged 22.4 and 11.7years had FIQ of 52 and 53, respectively. There was no statistical evidence of significant differences between the compliant, early-treated individuals and their unaffected siblings (controls) except for the FIQ, which was significantly higher than that of the unaffected siblings (P = .040)." "Statistical analysis: …..In 1 family of 3 children, 2 of whom were compliant patients, the same unaffected sibling was used as the control. There were 8 newborn-screened compliant patient-unaffected sibling control pairs used in the analysis. The remaining 2 were poorly compliant newborn-screened-unaffected sibling pairs." Note: The comparison should also have compared only between the 8 newborn-screened compliant patient-unaffected sibling control pairs, excluding those 2 poorly-compliant sibling pairs. The first sentence above is incorrectly worded in any case. In any case there is the possibility of confounding through parents and teachers being not blinded to the child's CBS condition and reacting to this with different volume/quality of teaching/other social factors. Also, tests of ss should have been done on various other pairs of groups, ie the 13 good-compliance with the 6 poorly-compliant, and reported. "Treatment regimens: All patients diagnosed with CBS-- were commenced on oral pyridoxine while on a normal diet, to ascertain their clinical responsiveness to pyridoxine. Once pyridoxinje-nonresponsiveness was confirmed, as indicated by a persistently high or rising plasma methionine and free homocystine, the patient was commenced on dietary management of methionine restriction and a methionine-free cystine-supplemented synthetic amino acid mixture. Plasma VitB12 and folate were assayed and if these were found deficient the patient was given supplements. Betaine, a remethylating agent, was used in the last 5years as an adjunct to treatment only in those patients (late adolescent/young adults) who became poorly compliant to dietary management (Yap and Naughten 1998). In the late-detected pyridoxine-nonresponsive patients, betaine was started with cofactor (pyridoxine, VitB12 and folate) supplementation. This regimen of betaine and cofactors did not seem to lower the free homocystine and methionine concentrations (this would be very unexpected regards the homocystine – perhaps they meant "sufficiently" DV) in our patients and the addition of dietary methionine restriction with supplementary methionine-free amino acid mix was necessary to obtain biochemical control."
Kalkanoglu HS et al (2001); J Inherit Metab Dis; Hacettepe U, Ankara, Turkey.	6 CBS--	"Summary:... We analysed 6 CBS-- for FVLeiden and prothrombin G20210A mutations. Only 1 patient was found to have the FVLeiden mutation in homozygous form and this patient had suffered from severe thrombosis. 1 patient was found to be heterozygous with no documented thrombosis. None of the patients had prothrombin G20210A mutation." Note: There was in fact another of the 6 patients, who had experienced deep vein thrombosis, who was homozygous wild-type for FVLeiden. No treatment details whatsoever provided.
Sokolova J et al (2001); Hum Mutat; Charles U, Prague, Czech Republic.	Pop Prev's of CBS--	(abstract) "….First, the incidence of CBS-- estimated by selective biochemical screening in the Czech and Slovak Republics was 1:349,000…two most common pathogenic mutant alleles…..IVS11-2A>C calculated prevalence 1:2,381; c.833T.C 1:5,556. Second…1284 unselected newborns…. ….c.833T.C observed prevalence 1:513; IVS11-2A>C not detected amongst 2,568 newborns. The estimated incidence of CBS-- of 1:83,000, calculated in a combined model, suggests that selective biochemical screening may ascertain only approx 25% of all CBS--. In conclusion, CBS-- in Central Europe may be sufficiently common to consider sensitive newborn screening programs for this disease." Note: Differential fetus viability purportedly seems not causing/contributing to this difference.

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Gaustadnes M et al (2002); Hum Mutat; Prince of Wales Hospital, Randwick, The Children's Hospital, Sydney, Royal Brisbane Hospital, Brisbane, Women's and Children's Hospital, Adelaide, all Australia.	36 CBS--, 13 VitB6-responsiv 2 VitB6-partially-responsiv 21 VitB6-nonrespon	"We determined the molecular basis of CBS-- in 36 Australian patients from 28 unrelated families, using direct sequencing of the entire coding region of the CBS gene." Sib Pat EthnicOrigin Allele1 Allele2 VitB6resp AgeD Diagnosis Lent Retard Other 1 1 Anglo-Celtic I278T I278T responsive 20 Osteopor no no PectCarin 1 2 Anglo-Celtic I278T I278T responsive 17 FamilScr yes no 2 3 Anglo-Celtic I278T L101P responsive 13 EctLentis yes no 2 4 Anglo-Celtic I278T L101P responsive 11 FamilScr no no 3 5 Anglo-Celtic I278T C109R responsive 14 EctLentis yes no 4 6 Anglo-Celtic I278T IVS8+1G>A responsiv 14 Retard,Tall no mild 5 7 Anglo-Celtic I278T IVS1+insC responsive 12 EctLentis yes no Marfanoid 5 8 Anglo-Celtic I278T IVS1+insC responsive 14 FamilScr no no 6 9 Anglo-Celtic I278T 1221delC responsive 11 FamilScr no no 7 10 Anglo-Celtic I278T G347S,T191T respons 9 EctLentis yes no 8 11 Anglo-Celtic I278T undetermined respons 8 EctLentis yes mild Osteopen 9 12 Anglo-Celtic E144K P49L responsive 4 FamilScr no no 10 13 Anglo-Celtic E144K V371M partially 8 EctLentis yes border PectExc 11 14 Anglo-Celtic I278T T353M partially 29 EctLent,Skel y no Kyphoscol 12 15 Anglo-Celtic G307S G307S nonrespons 3 EctL,Retard y mild 13 16 Anglo-Celtic G307S G307S nonrespons 3 EctL,Marfan y mild Marfanoid 14 17 Anglo-Celtic G307S G307S nonrespons 4 EctLentis yes no 15 18 Anglo-Celtic G307S G307S nonrespons 7 EctL,Retard y moder Scoliosis 15 19 Anglo-Celtic G307S G307S nonrespons 23 FamilScr no no TIA(?DV) 16 20 Anglo-Celtic G307S C165Y nonrespons 7 EctLentis yes no Scoliosis 17 21 Anglo-Celtic G307S R336C,A335A nonresp <1 NewbornScr no no 18 22 Anglo-Celtic G307S 533del18 nonrespons 7 EctL,DevDel y mild Marfan 19 23 Anglo-Celtic E144K IVS1+1G>A nonresp 5 EctLentis yes mild Kyphoscol 20 24 Anglo-Celtic E144K,R439Q E320K nonrespons 6 EctLentis yes no Marfanoid 21 25 Anglo-Celtic E144K,R439Q 829insC nonrespons 9 EctL,DevDel y mod sevOstPor 22 26 Anglo-Celtic A331E 442insG nonrespons 2 DevDel,OstImp y sev sevOstPo 23 27 Anglo-Celtic N228K IVS11+1G>A nonresp 6 Marfanoid yes mild Marfan 24 28 Anglo-Celtic C109R undetermined nonresp 12 EctLentis yes border 25 29 Anglo-Celtic C109R 533del18 nonrespons 1 DevDel no mild 25 30 Anglo-Celtic R369C 533del18 responsive 24 FamScr,DVT no no PulmEmb 26 31 Portugese R125Q R125Q nonrespons <1 NewbornScr yes no 26 32 Portugese R125Q R125Q nonrespons 2 FamilScr yes mild SternDef 27 33 Sth African C165Y 1622insTGAA nonresp 41 EctL,Skel yes no 28 34 Lebanese 19insC 19insC nonrespons 5 AbnMethotrex y border 28 35 Lebanese 19insC 19insC nonrespons 9 FamilScr no no 28 36 Lebanese 19insC 19insC nonrespons 1 FamilScr no no Where: Sib = Sibship #, Pat = patient #, EthnicOrigin = ethnic origin, Allele1 = one allele, Allele2 = the other allele, VitB6resp = VitB6-responsivity, AgeD = age at diagnosis, Diagnosis = phenomena at diagnosis Lent = Ectopia Lentis, Retard = mental retardation, Other = other features, &, EctLentis, EctL = Ectopia Lentis, Y = yes, DevDel = developmental delay, mod, moder = moderate, sev = severe, Marfan = Marfanoid, responsiv, respon = responsive, nonrespons, nonresp = nonresponsive, NewbornScr = newborn screened, FamilScr, FamScr = family screened, Kyphoscol = kyphoscoliosis, sevOstPor = severe osteoporosis, SternDef = sternal defect, OstImp = Osteogenesis Imperfecta, PectCarin = Pectus Carinatum, PectExc = Pectus Excavatum, Skel = skeletal PulmEmb = pulmonary embolism, TIA = ?, AbnMethotrex = abnormal Methotrexate response

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Levy HL et al (2002); J Inherit Metab Dis; Various USA & Canada.	11 CBS--Mothers, & offspr. Family#	Note: No offspring homocystinuric VitB6Resp AgeDiagn PregComplic CongenAnom OAge OIQ	Maternal tHcy during gestation	& treatment start t
	1	Nonrespon 7years ToxemiaDelivery ColobIrisChor, UndescTestis,NTD 14y IQ60	>170uM	12weeksG VitB6 + folate + betaine
	2	Nonrespon newborn BleedTrim1&2 none 4y IQ103	111uM, 5uM	10weeksGLMetD; 12weeksGVitB6 + folate + betaine
	3	Nonrespon 6years UTI,borderHT none 5y IQ90	279uM	VitB6 + folate
	4	Nonrespon 4.5years none none 7y IQ109	208uM	VitB6 + folate + betaine; aspirin, heparin, coumadin
	5	Nonrespon 6years SpontAbort	222uM	VitB6 + folate + betaine; aspirin
	6 Preg1	Responsive 32years SpontAb none 7y IQ121		Nil
	Preg2	none Beckwith-Wiedemann: 5y IQ90 Macroglossia,PulmValveStenosis, Hemihypertrophy. AutDom?		
	Preg3	ElevAFP,ThromPhl		
	7	Responsive 25years none none 8y IQ104	120uM	VitB6 + from 25weeksGVitB12
	8 Preg1	Responsive 23years UTI none 7y IQ113	6uM	VitB6
	Preg2	HT none 5y IQ101		
	9 Preg1	Responsive 17years ElectAb none 2.5y DQ108	9-36uM	VitB6 + folate; aspirin
	Preg2	none		
	10	Responsive 11years none none 1.3y DQ111	9-17uM	VitB6 + folate; aspirin
	11	Responsive 5years none none 0.7y DQ98	15-30uM	16weeksG LMetD; VitB6 + folate + from 32weeksG betaine
		Where: VitB6Resp = VitB6-responsivity, AgeDiagn = age of CBS--diagnosis, PregComplic = complications of pregnancy, CongenAnom = congenital anomalies, OAge = offspring's age, at which OIQ = offspring's IQ/DQ, ColobIrisChor = coloboma of iris and choroid, UndescTestis = undescended testes, NTD = neural tube defect, HT = hypertension, UTI = urinary tract infection, SpontAb = spontaneous abortion, ElectAb = elective abortion, ThromPhl = thrombophlebitis, LMetD = low-methionine diet		
	NormVal		7.9(2)uM	

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Pullin CH et al (2002); J Inherit Metab Dis; Various, UK	5 CBS--, & controls					tHcy at study entry	
	5 CBS--:		AgeD/T	Age	VitB6Resp		
	CM		5y	23y	Responsive	47uM	VitB6 + folate + betaine
	DF		15y	25y	Responsive	36uM	VitB6 + folate + betaine + LMetD
	AC		21y	46y	Responsive	141uM	VitB6 (increased 600+% from 40mg/d fr week7) + folate + VitB12
	CA		NewbornScr	16y	Nonrespons	96uM	VitB6 + folate + betaine
	LW		2y	21y	Nonrespons	181uM	VitB6 + folate + betaine + LMetD
		Where: AgeD/T = age at diagnosis/treatment, Age = age at time of study, VitB6Resp = VitB6-responsivity, LMetD = low-methionine diet "Methods. Subject Recruitment: The patient group comprised 5 individuals (1 male, 4 female) aged 16-46years (mean age 26y) with CBS--, all of whome were receiving Hcy-lowering treatment…. None of the patients recruited to the study smoked. Age and gender-matched controls were recruited from workplace staff, and were specially selected to be free from cardiovascular risk factors including hypertension (diastolic BP<80mmHg), diabetes, smoking and hypercholesterolemia (total chol<6.2mM). Plasma tHcy was within the normal range (5-15uM) and subjects were not taking vitamin supplements….. Study design: The study was divided into 2 phases designed to investigate the vascular effects of both acute and chronic administration of high-dose VitC (500mg tablets, Boots plc). Subjects were required to attend on 3 study days. Baseline investigation: (Study day 1) Following an overnight fast, blood was sampled and brachial artery responses to increased blood flow, ie endothelium-dependent FMD, and sublingual nitroglycerin (NTG, 400ug) administration (endothelium-independent dilatation) were assessed. Acute effects of vitC: (Study day 1) Following baseline assessment, subjects were administered 2g oral VitC in a single dose. Blood was sampled and brachial artery FMD was reassessed 1, 2, 3 and 4h following VitC ingestion. Endothelium-independent responses were not assessed so as to avoid nitrate carry-over effects. Subjects were given a standardized low-fat, low-protein sandwich and caffeine-free drink between the baseline and 1h assessments. (Continues)					
(Continues)							

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(Continued) Pullin CH et al (2002); J Inherit Metab Dis; Various, UK	(Contin) 5 CBS--, & controls	(Continued) Chronic effects of VitC: Subjects provided a fasting blood sample and underwent reassessment of brachial artery responses following 2weeks' administration of VitC in a single dose (2g/day) (study day 2), and after 6months' administration of VitC in a single dose (1g/day) (study day 3) (numeration of study days seems peculiar/poor/incorrect. DV). Noninvasive measurements of vascular endothelial function: …..Brachial artery end-diastolic diameter (EDD) was measured by high-resolution (+/-3um) ultrasonic vessel 'wall-tracking' (Vadirec), blood flow by continuous wave Doppler, and blood pressure by photoplethysmography (Finapres). Following baseline measurements, a wrist cuff was inflated to suprasystolic BP. Inflation of this cuff for 5min induced ischemia in the hand. Release of the cuff stimulated reactive hyperemia in the hand, resulting in a secondary increase in blood flow along the brachial artery. EDD, blood flow, and pressure were recorded at 1, 2, 3, and 5min after cuff release, and again at 10min to demonstrate vessel recovery. FMD was taken as the maximum absolute change in EDD (um) observed during the first 3min after cuff release. Then, as appropriate (baseline only on study day 1, and study days 2 and 3), EDD, blood flow and pressure were measured 3min after 400ug sublingual NTG administration."	
		Diam FMD NTG SystBP DiastBP HR VitC 5-MeTHF Met tCys	tHcy
	CBS-- : Baseline;	3.2mm 20 ***vC 388 114mmHg *vC 72 63bpm 55uM *vC 72nM ***vC 440uM ***vC 139uM ***vC	101uM
	2weeks;	170 ***vB 450 118mmHg 72 65bpm 120uM 1***vB 75nM 1***vC 436uM 1***vC 165uM *vC	85uM
	6months	170 ***vB 390 125mmHg 1*vB 71 67bpm 95uM 1**vB 78nM 1***vC 241uM 1***vC 1***vB 173uM *vC	95uM
	Controls: Baseline;	3.2mm 116 470 127mmHg 72 63bpm 89uM 23nM 25uM 248uM	9uM
	2weeks;	140 450 116mmHg 65 63bpm 120uM 27nM 22uM 232uM	9uM
	6months	130 450 125mmHg 68 63bpm 105uM 25nM 23uM 264uM	9uM
		Where: Diam = as detailed above, FMD = Flow Mediated Dilatation, NTG = arterial response to nitroglycerin, SystBP = systolic blood pressure, DiastBP = diastolic blood pressure, HR = Heart Rate, VitC = vitamin C, 5-MeTHF = 5-methyl-tetrahydrofolate Met = methionine, tCys = total plasma cysteine Note: some of the comparisons 'twixt CBS—and controls only considered ss to the level of P<.05, and not to any level of ss greater than this, so some of those comparisons may in fact be more ss ie P<.01, <.001, but are unable to be noted here as such….while others (ie methionine) were obviously more ss than noted, and I have taken the liberty of adjusting my reporting of the ss accordingly, denoting these by placing a "l" before any such asterisk(s). Note: In properly multivariate considerations, the change in methionine and cysteine, conjuntively indicating better compliance with dietary recommendations, assuming some other improvement in that metabolism has not taken place, and the increase in systolic blood pressure, all these in the CBS--, ought be adjusted for, which would reduce the apparent effect associated with the VitC treatment, though very probably not so much as to make it nss.	

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Authors, Journal,	Study Group	Disease	Hcy level
Atalay S et al (2002); Acta Paediatr; Ankara U, Turkey.	13 consec intracard thrombos	(Abstract) "The objective of this study was to determine the association between intracardiac thrombosis and heridatary causes of thrombophilia, including factor V 1691 G-A (factor V Leiden, FVL) and prothrombin 20210 G-A mutations. Over a period of 3years, genetic risk factors were evaluatedin 13 consecutive children (mean age 6.27+/-5.44y) with intracardiac thrombosis, diagnosed by cross-sectional echocardiography. Thrombi were localized in the left heart in 4 patients and the right heart in 9 patients. All children had predisposing conditions for thrombus formation: ventriculoatrial shunt for hydrocephalus (n = 3), indwelling catheter for chemotherapy (n = 5), cardiomyopathy (n = 2), sepsis (n = 1), homocystinuria (n = 1), and tetralogy of Fallot (n = 1). 6 of the 13 children with intracardiac thrombosis were heterozygotes for FVL mutation. 3 of these 6 children had ventriculoatrial shunt for hydrocephalus, 2 had cardiomyopathy, and 1 had sepsis. None of the children carried the prothrombin 20210 G-A mutation." (Note: as inferred, the homocystinuria (CBS-- ?) case did not carry the FVLeiden mutation)	
Kruger WD et al (2003); Hum Mutat; Fox Chase Cancer Center, Philadelph., Pennsylvan. USA.	12 CBS—from 11 different families, from Georgia, USA, including 4 African-American	(abstract) "….By DNA sequencing of all the coding exons we identified mutations in in the CBS genes in 21 of the 22 possible mutant alleles. 10 different missense mutations were identified and 1 novel splice-site mutation was found. 5 of the missense mutations were previously described (G307S, I278T, V320A, T353M, and L101P), while 5 were novel (A226T, N228S, A231L, D376N, Q526K)…. The I278T and T353M mutations accounted for 45% of the mutant alleles in this patient cohort. The T353M mutation, found exclusively in 4 African American patients, was associated with a VitB6-nonresponsive phenotype and detection by newbornm screening for hypermethioninemia. The I278T mutation was found exclusively in Caucasian patients and was associated with a VitB6-responsive phenotype." AgeD VascEv **tHcy** EctLent AgeD VascEv **Met** EctLentis 12y yes 210uM yes Birth no 2000uM no 13y no 196uM no Birth no 1181uM no 21y no 187uM yes Birth yes 835uM no 12y no 186uM yes Birth no 831uM no 6y yes 174uM yes 6y no 603uM yes 20y no 171uM yes 13y no 578uM no 22y yes 169uM yes 12y no 504uM yes Birth no 155uM no 20y no 418uM yes Birth no 152uM no 22y yes 256uM yes Birth yes 150uM no 6y yes 111uM yes Birth no 118uM no 12y yes 105uM yes Note: The LHS and RHS contain the same cases, but that the one is ordered according to the tHcy level, the other is ordered according to the mehtionine level. Where: AgeD = age at diagnosis, VascEv = had a vascular (thrombotic) event, **tHcy =** pre-treatment plasma tHcy, derived from Hcy(ine) given using algorithm of Bonham et al (1997), such that: tHcy = 60 + 4.5(fHcy(ine)) for values of fHcy(ine) < 20uM, and tHcy = 60 + 90 + (fHcy(ine) – 20) for values of tHcy(ine) >=21uM, **Met** = pre-treatment plasma methionine, EctLent = Ectopia Lentis, Birth = newborn screening First note the ascertainment bias resulting from diagnosis by raised methionine in the case of the newborn screening. In any case, they may be safely excluded from the following considerations, which in any case do not approach ss. Vascular events are associated with Ectopia Lentis; the ss of course is negligible, and the ascertainment bias of Ectopia Lentis probably being the presentation trigger to test for CBS-- precludes much/any meaning being derivable from this. Vascular events are not associated with tHcy level, here. Vascular events are associated with lower methionine levels, here. The latter two observations are not in accord with generally accepted extancies.	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Kelly PJ et al (2003); Neurology; Massachu-setts General Hospital, Boston, Massachu-setts, USA.	3CBS--without usual stigmata	"Patient A was a 47y-old white woman of northern European descent who presented with acute left-sided weakness. She was of normal intelligence, with a history of hypertension and tobacco use. Family history was unremarkable for premature atherosclerosis, thrombosis, or intellectual impairment…. left hemiparesis (MRC grade 4/5), hemisensory impairment, and extinction to tactile stimuli, without skeletal abnormalities or lens displacement…. (imaging DV) consistent with acute infarction…. hypoechoic mass in the proximal right internal carotid artery consistent with thrombus… .but no evidence of extra- or intracranial atherosclerosis…. Serum and red cell folate, VitB6, VitB12, lipids, hypercoagulability screen (protein C, protein S, antithrombin 3, anticardiolipin antibodies, prothrombin gene 20210 polymorphism), transthoracic echocardiography, and 24hour Holter monitoring results were normal. …Repeat B-mode carotid ultrasound at 5weeks showed complete resolution of the of the intraluminal signal abnormality, consistent with dissolution of thrombus… Marked improvement occurred, with resolution of the sensory deficits and minimal residual weakness at 6months…. heterozygous for T833C (I278T aa substitution). Severe hyperhomocystinemia is rare (ever? DV) in T833C heterozygotes, suggesting compound heterozygosity for another unidentified CBS mutation on the other allele….. MTHFR C677T was homozygous wildtype. The asymptomatic 46y-old sister…..heterozygous CBS T833C, MTHFR C677T was homozygous wildtype. Patient B was a 39y-old Venezuelan man presented with a right frontal headache and acute visual loss in the right eye. Medical history was unremarkable for neck trauma or vascular risk factors. Examination revealed an afferent pupillary defect, reduced visual acuity, and superior altitudinal field defect in the right eye. Fundoscopic examination revealed edema in the territory supplied by the inferior temporal branch of the right central retinal artery. Neurologic and general examination findings were otherwise normal, although he was noticeably tall and thin. CT scan of the brain was normal. Cerebral angiography was unremarkable except for a tapering "flamelike" occlusion of the right ICA at the origin, with "double density" of contrast at the site of occlusion, suggesting pooling of dye in the space created by an intimal flap. Together with the clinical presentation , these findings were strongly suggestive of ICA dissection. Mild scoliosis was present on chest radiography…. cultured skin fibroblasts…(heterozygous G1330A (D444N aa substitution)… partial response to pyridoxine….FVLeiden not detected….. The patient was followed clinically for 17years, without recurrent ischemic events. (Continues)	tHcy 279uM 8uM (? Seems too low) 128uM 16.5uM 212uM 43-102uM	Met = 125uM Warfarin; VitB6 300mg/d, Folate 1mg/d Met = 60uM VitB6 300mg/d, VitB12 80ug/d Met = 58uM Aspirin; VitB6 200mg/d, Betaine 4.5g/d
(Continues)		(Continues)		

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
(Continued) Kelly PJ et al (2003); Neurology; Massachu-setts General Hospital, Boston, Massachu-setts, USA	3CBS-- without usual stigmata	(Continued) An asymptomatic sister…. 5'8" tall with mild scoliosis of the thoracic and lumbar spine. There was no clinical evidence of ocular, intellectual, or vascular manifestations…… (heterozygous G1330A (D444N aa substitution). Patient C was an 18y-old man of Irish ethnicity with known CBS--, referred for evaluation of abrupt deterioration of school mathematical ability, which was nonprogressive and had begun acutely 4months previously. Medical history was remarkable for mitral valve prolapse (MVP) and severe hyperhomocysteinuria detected 1year earlier, unresponsive to supplemental vitamins and dietary methionine restriction. There was a strong paternal family history of premature coronary artery disease and a maternal family history of aortic dissection, but no family history of stroke or intellectual impairment…. tall thin man with mild pectus excavatum. Neurologic examination was normal. An abnormal focus of FLAIR MRI hyperintensity was present in the right parietal subcortical white matter, consistent with ischemic stroke. MRI and MRA of head and neck showed no signal abnormality suggestive of dissection, atherosclerosis, or venous thrombosis (normal venous flow voids on T1 MRI). …. Holter cardiac monitoring, lipid and hypercoagulability profiles (anticardiolipin antibodies, proteins S and C, antithrombin 3, lupus anticoagulant assay, FVLeiden) were normal. Neuropsychological evaluation revealed impaired retention of new information and impaired ability to perform geometric constructions and other visual-spatial tasks, consistent with right parietal lobe lesion…. G919A (G307S aa substitution)… Despite therapy (as noted column right DV) tHcy remained elevated 2years later." "First, they support the rationale for screening for subclinical hyperHcy in young adults with premature stroke, despite the apparent absence of classic phenotype features of CBS--, such as ectopia lentis, intellectual impairment, seizures, and skeletal abnormalities… Secondly…….may also promote embolic stroke of cardiac and arterial origin, possibly in individuals otherwise predisposed to thrombophilia by conditions such as cardiac structural abnormailities, atrial fibrillation, and FVLeiden."	91uM 62-85uM 175uM 281uM	Met = 98uM VitB6 300mg/d Met = 821uM, Normal folate, VitB12 Low-Met diet; VitB6, folate, VitB12 (com-pliance?)
Yap S (2003); J Inherit Metab Dis; Children's University Hospital, Dublin, Ireland.	158 CBS-- Irish, Australian Dutch and UK.	This paper does not seem to add anything to the contents of Yap et al (2001a), see above.		

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Orendac M et al (2003); J Inherit Metab Dis; Charles U, Prague, Czech Republic.	9 or 5 CBS--, Czech	"Summary : To explore the pathogenesis of CBS-- and to test the efficacy of pharmacological therapy we examined a panel of metabolites in 9 CBS-- patients under treated and/or untreated conditions. Off pharmacological treatment, the biochemical phenotype was characterized by accumulation of tHcy (median 135uM) and blood S-adenosylHcy (median 246nM), and by normal levels of guanidinoacetate and creatine. In addition, enhanced remethylation was demonstrated by low serine level (median 81uM), and by increased concentration of methionine (median 76uM) and N-methylglycine (median 6.8uM). Despite the substantially blocked transulfuration, which was evidenced by undetectable cystathionine and severely decreased cysteine levels (median 102uM), blood glutathione was surprisingly not depleted (median 1155uM). In 5 patients in whom pharmacological treatment was withdrawn….(see table below DV)… In summary, our study shows that conventional treatment of CBS-- by diet and pyridoxine/betaine normalizes many but not all metabolic abnormalities associated with CBS--. We propose that the finding of low plasma serine concentration in untreated CBS-- patients merits further exploration since supplementation with serine might be a novel and safe component of treatment of CBS--." The subset of 5 patients whose data comprise the tabulation further below: Age VitB6resp Mutations StudyWashout	tHcy	Pre-washout
	#2	10y nonrespons AIVS11-2C + (c.G430A + c.G463A) Completed		VitB6, folate, betaine
	#3	14y nonrespons AIVS11-2C + (GIVS7+1A + IVS11+39del99) "		VitB6, folate, betaine
	#7	20y responsive c.C341T + c.G1226A Completed		VitB6, folate, betaine
	#8	25y responsive c.T833C + AIVS11-2C (partially)Completed		VitB6, folate,
	#9	32y responsive c.T833C + c.T833C Completed		Untreated, non-compliant
		Note: Completion of the study washout period was the criteria for data contribution to the table below – the other 4 patients did not complete, and therefore no data is included from them. The partial completion of washout noted lasted 14days until abandoned due to increase of tHcy, on decision by unknown patient/practitioner/scientist/protocol.		Note: All compliant were on low-Met diet through-out whole study
(continued)		(continued)		

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
(Continued) Orendac M et al (2003); J Inherit Metab Dis; Charles U, Prague, Czech Republic.	(Cont) 9 or 5 CBS--, Czech	(Continued) Metabolite — RefRange — CBS--Treatment — CBS--OffTreatment — P tHcy — 5.4-13.9uM — 32.7 (6.6-65.3)uM — 125 (19-135)uM — .009 SAH — 12-99nM — 59 (21-162)nM — 132 (47-350)nM — .06 Met — 13-43uM — 26.3 (17-156)uM — 76 (16-78)uM — 0.75 SAM — 1.2-2.3uM — 1.8 (1.4-2.0)uM — 1.9 (1.5-2.4)uM — 0.16 SAM/SAH — 20-118 — 29 (12-86) — 14 (5-43) — .06 Cystathionine — 50-342nM — subdetection — subdetection — n.a. tCys — 200-360uM — 211 (137-258)uM — 139 (102-192) — .05 Guanidinoacetate — 1.67-4.0uM — 2.7 (1.9-3.6)uM — 2.8 (1.7-4.0) — 0.92 Creatine — 16.4-58uM — 20.1 (16.4-36.1) — 20.7 (16.7-33.3) — 0.32 N-methylglycine — 0.6-2.7uM — 12.7 (3.8-38)uM — 6.8 (3.2-8.9)uM — 0.15 N,N-dimethylglycine — 2-6.6uM — 38.0 (3.5-275.5)uM — 2.9 (2.1-5.2)uM — 0.16 ???? Serine — 97-267uM — 103 (69-111)uM — 53 (50-81)uM — .036 Glycine — 152-413uM — 234 (165-295)uM — 226 (188-361)uM — 0.25 Glutathione — 790-1,350uM — 1290 (980-1,580)uM — 1160 (940-1,230)uM — 0.15 Where: SAH = S-adenosylHcy, Met = methionine, SAM = S-adenosyl-methionine, tCys = plasma total cysteine Note: although ideally there should be adifferentiation on the basis of VitB6-responsivity, their figure with all individual measurements plotted does not indicate much difference between these. "On day 1, the pharmacological therapy was discontinued, and patients were instructed to continue the low-methionine cystine-supplemted diet (M-AM, SHS International)." Apparently the diet was continued throughout. This makes it difficult to assess the meaning of the data, in particular to relate it to reality where it is more likely that compliance will be achieved with the pharmacological than the dietary treatments. "Oral glucose tolerance test:…..In conclusion we did not find any gross signs of impaired endocrine pancreatic function in CBS--." "Markers of endothelial dysfunction: We examined whether patients with CBS-- exhibited abnormalities in factors of the thrombolytic system (tPA and PAI-1) and signalling molecule ICAM-1, all of which are considered markers of endothelial dysfunction. There was no correlation between tHcy and plasma ICAM-1 concentrations. In contrast, there was a strong correlation between tHcy and tPA concentrations (r2 = 0.51, P = .0018), and between tHcy and PAI-1 concentrations (r2 = 0.48, P = .0029). These data suggest that severe hyperHcy impairs endothelial function."

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
Linnebank M et al (2003); J Inherit Metab Dis; U Munster, U Bonn, Germany.	Stroke and sinus thromb-osis patients, apparently unselected ?....	"…DNA samples of patients with stroke (n = 225) and sinus thrombosis (n = 46) were screened for the most common homocystinuria mutation, CBS I278T…. The frequency of I278T heterozygosity (0 of 46 patients with sinus thrombosis and 2 of 225 patients with stroke) was not elevated in comparison to the healthy German population (Linnebank et al 2001). Therefore, this study does not provide any hint of CBS-+ as a risk factor for thrombophilia. However, 2 individuals were found to be of homozygous allele state for I278T…. In the stroke group, there was a homozygous 34y-old male with bilateral occlusion of the carotid arteries and of one vertebral artery, and with 3 previously documented stroke episodes. In the sinus thrombosis group, a 24y-old woman, who developed symptoms after delivery of a healthy daughter via Caesarian section, was of a homozygous allelic state. Several further risk factors relevant for thrombosis such as MTHFR C677T and A1298C, prothrombin G20210A, plasminogen inhibitor polymorphisms, APC resistance, proteins C and S, lipoprotein(a), and antiphospholipid antibodies were investigated. The stroke patient was heterozygous for APC resistance, which leads to an additional 4- to 5-fold increased risk of recurrent thrombosis (McKusick 227400); the patient with the sinus thrombosis was without respective risk factors…….. (I278T is VitB6-responsive DV)……. the 2 CBS--in this report did not present with any other nonvascular symptoms of homocystinuria."
Sueyoshi E et al (2004); AJR; Nagasaki U, Nagasaki, Japan.		"A 21y-old man was admitted to our hospital with a 3year history of intermittent claudication. Arteriography of the lower extremities showed stenosis of both external iliac arteries. Venography of the lower extremities showed thrombus in the left poplitiael vein and development of collateral vessels. Initially the patient was treated with urokinase and antiplatelet agents. 13days after admission, he complained of chest pain and dyspnea. A pulmonary perfusion scintigram revealed multiple perfusion defects in both lungs, and the diagnosis of pulmonary embolism was made. After treatment with urokinase and antiplatelet agents, the symptoms of pulmonary embolism gradually subsided. (Is this implying that the thrombus was released from the popliteal vein to relocate to the lung?...seems quite possible….DV)…. (hyperHcy and VItB6-responsive CBS-- established DV)…. After the patient underwent treatment with VitB6, his coagulability and Hcy returned to normal. (Note: In 1980 when this was assayed, often fHcy, not tHcy, was assayed, and the former may be undetectable at tHcy of up to 40uM DV). The patient was discharged and treated with a supplement of VitB6 as an outpatient. He had remained symptom-free after discharge, and follow-up arteriography and venography of the lower extremities showed no abnormality. However imaging studies of the lung showed a gradual increase in the size of segmental branches of the pulmonary arteries. Pulmonary ventilation-perfusion scintigrams showed multiple newly developed mismatched defects in both lungs. Fortunately there have been no symptoms related to chronic pulmonary embolism. His pulmonary arterial systolic pressure has slightly increased, but remained below 90mmHg during the follow-up period. The patient is still doning well after 25years of follow-up." Note: None of the other common stigmata of CBS-- extant?.... treatment with VitB6 **alone** (assume?....) means the Hcy is Going down the CBS pathway, providing both cysteine (ie for fibrillin) and sulfate (ie for heparin sulfation) – so, is some other connective tissue disorder overlayed on the CBS--?..... What was compliance?....

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Singh RH et al (2004); Genet Med; Emory U, Atlanta, Georgia, USA.	5 CBS--	"5 patients with biochemically confirmed CBS-- cared for by the Division of Medical Genetics at Emory U were recruited for this study…….3 of the 5 patients were identified by positive newborn screening for hypermethioninemia, and 2 were identified later in life after the discovery of ectopia lentis. All patients required methionine restriction to <30mg/kg/day, to decrease their free Hcy and total Hcy. Met-restricted diets were implemented concurrently with VitB6 supplemetation at doses up to 20mg/kg/day. We selected for this study 5 patients classified VitB6-nonresponsive. All were treated with methionine restriction and pharmacological supplementation of VitB6, but could not maintain plasma methionine below 50uM or fHcy below 5uM (tHcy<45uM DV). The treatment of VitB6-nonresponsive CBS-- patients with "methionine restriction" was accomplished in part by the simultaneous dietary restriction of natural protein, and supplementation withy medical foods free of methionine. These Met-free medical foods were supplemented with conditionally-essential l-cysteine, in the form of l-cystine. Study Design. Patients were first treated with natural protein restriction, supplementation with Met-depleted, l-cystine-fortified medical foods, and 5 to 20mg/kg/day of pyridoxine. Upon attaining the lowest plasma methionine and tHcy, we began betaine at prescribed doses of 20-50mg/kg/day. Dosages were increased to 120-150mg/kg/day and provided as 3 divided doses until stabilization of tHcy to the lowest concentrations for the individual. Biochemical measurements were obtained every 1-3months, until the tHcy reached a nadir. Three-day diet histories were analysed and compared for methionine content, and other essential nutrients throughout the studies….. All patients were classified VitB6-nonresponsive by clinical criteria."	tHcy	
	#, Age	Ethnic Mut1 Mut2 MetPre MetBet tCysPre tCysBet tHcyPre	tHcyBet	
	#1, 1y	Afr-Am T353M Q526K 24uM 116uM 109uM 118uM 28uM	7uM	
	#2, 10y	Caucas V320A V320A 21uM 38uM 203uM 150uM 38uM	6uM	
	#3, 11y	Caucas I278T D376N 12uM 21uM 194uM 217uM 31uM	11uM	
	#4, 18y	Caucas I278T L101P 123uM 103uM 138uM 284uM 135uM	31uM	
	#5, 19y	Afr-Am T355M T355M 69uM 140uM 97uM 144uM 119uM	60uM	
	All	Average Change (P): +34uM(P=0.18) +34uM(P=0.35) -47uM	(P=.02)	
		Where: Ethnic = ethnicity Mut1,Mut2 = the two CBS- mutations, MetPre, MetBet = methionine before, and with, betaine, tCysPre, tCysBet = plasma total cysteine before, and with, betaine, tHcyPre, tHcyBet = tHcy before, and with, betaine. However, note: "Although we observed 4/5 patients with a near doubling of their plasma methionine during maximal betaine therapy, the mean plasma methionine in our betaine treatment group did not change. This was because of the decrease in the plasma methionine of patient 4, ….. this patient reported improved compliance with both methionine restriction and consumption of the methionine-depleted, cystine-enriched medical food…..evidence supporting improved compliance by patient 4 was the doubling of his total plasma cysteine and the decrease in plasma methionine during betaine treatment, which was only observed in this subject. This patient reported being partially compliant with his betaine dosing regimen, only taking one fourth of the prescribed dose (approx 20-30mg/kg/day)…..Of interest was that his ratio of Met/tHcy increased as in other patients indicating that remethylation of Hcy was enhanced by betaine.		

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level
Devlin AM et al (2004); J Pediatr; Newcastle General Hospital, Newcastle-upon-Tyne & Willink Bio-chemical Genetics Unit, Royal Manchester Children's Hospital, Manchester, Both UK	1 CBS--	(abstract) "….Betaine is formed naturall in choline metabolism. It is involved in the maintenance of cell volume against osmotic stress and acts as a chemical chaperone, protecting proteins against denaturation. It is also a substrate for the hepatic enzyme betaine-homocysteine methyltransferase. For >20years, pharmacological doses of betaine have been used to lower homocysteine concentrations in homocystinuria. The usual dose in children has been 250mg/kg/day, but recent studies suggest that there is little benefit from increasing >150mg/kg/day (cites Matthews et al 2002). The response to betaine is variable. Plasma homocysteine concentrations often fall by >=50%. Plasma methionine concentrations usually rise, sometimes only slightly but sometimes as high as 1000-1200uM. There have been few clinical problems, but cerebral edema was reported in a patient treated with betaine (cites Yaghmai et al 2002). We report a second patient with CBS-- who had this complication while receiving betaine." "Case History. A 4.5year-old boy was diagnosed with homocystinuria after presenting with dislocated lenses. He had poor speech articulation, clumsiness, and frequent falls, but development was otherwise normal. Baseline plasma tHcy concentrations ranged from 334-430uM (normal 5-17uM). The plasma methionine concentration was 203uM (normal 10-54uM) with a normal VitB12 level and a normal urinary organic acid profile. CBS activity in fibroblasts was undetectable, with no change after the addition of pyridoxal-phosphate or SAM. Clinically, there was only a partial response to pyridoxine (500mg/day) and folic acid (5mg/day) with tHcy of 253-340uM. At age 5years, betaine was started and increased gradually to 3g/day (150mg/kg/day), and dietary protein was restricted (methionine intake 500mg/day) (VitB6 and folate continued?....DV). 4weeks after starting betaine, the patient began having morning headaches and vomiting, which increased in frequency and severity. 2weeks later, he presented to the hospital with papilledema but with no other neurological signs. Cranial MRI with magnetic resonance angiography and venography showed no evidence of sinovenous thrombosis. There was however a widespread abnormal signal from the white matter in both cerebral hemispheres. Lumbar puncture revealed an opening pressure of 48cm of CSF (normal <20cmH_2O. DV) but was otherwise normal and resulted in a marked reduction of symptoms. 4days later, symptoms returned and were relieved by a second lumbar puncture (opening pressure 47cmCSF). Aspirin, dexamethasone, and acetazolamide were started. The betaine dose was doubled to to 3g twice daily. After another 4days, symptoms worsened, and lumbar puncture showed an opening pressure >80cmCSF. Relevant plasma and CSF results are shown in the Table.	334-430uM pre-treatment 253-340uM on VitB6 500mg/d, Folate 5mg/day
		CSF: Betaine Met Plasma: Betaine Met N,N-DMG	tHcy
	This Case	6.6uM 235uM 98uM 1,190uM 64uM	239uM
	Normal Range	Mosby's- 1-5uM 18-73uM 10-54uM 1.4-5.3uM	5-17uM
	CBS--s on betaine	? ? 170-660uM <1,200uM 33-250Um	?
		4hours after the third lumbar puncture, the patient became hypertensive and bradycardic with a falling level of consciousness and unilateral pupillary dilatation. Computed tomography showed diffuse brain swelling with loss of the basal cisterns. The patient was intubated, ventilated, and given mannitol, after which bilateral frontotemporal decompressive craniotomies were performed. Invasive angiography revealed no evidence of sinovenous or corticovenous thrombosis. Betaine was discontinued. Ventilation and fluid restriction were continued for 48hours, after which the patient recovered consciousness. Neurological examination was normal at discharge 8days later and at 9months. Psychomotor development has progressed normally. Cranial MRI after 6months showed resolution of edema and white matter signal abnormalities. Satisfactory levels of tHcy (<50uM) and Met (43-111uM) achieved with low methionine diet (200mg/day) and Met-free amino acid supplements (assume Cys-supplemented?..)"	

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Vilaseca MA et al (2004); J Inherit Metab Dis; U Hospital St Joan de Deu, Barcelona, Spain.	2 CBS--siblings, VitB6-non-responsiv, pregnancy experienc of one	"The patient was the second daughter of healthy parents. One older brother died undiagnosed at the age of 4months and a younger one, diagnosed with homocystinuria (tHcy = 250uM on treatment with 300mg/day pyridoxine) has ectopia lentis with blindness, mental retardation, and skeletal abnormalities. The woman was diagnosed at the age of 1year owing to ectopia lentis and was treated with pyridoxine (300mg/day). At the age of 30, clinical reevaluation showed severe myopia, ectopia lentis, disc herniation (L5), venous insufficiency, Marfanoid habitus, thin skin, dry and thin hair, and emotional lability.	tHcy 250uM	VitB6 300mg/d
		Biochemical data on 300mg/day pyridoxine treatment showed tHcy 180uM….Met 80uM and cystine (not cysteine DV) undetectable. Treatment was modified by increasing pyridoxine to 600mg/day, and by adding folate 10mg/day, cystine 0.5mg/day and betaine 6g/day. tHcy decreased to 56uM and Met to 55uM, while cystine increased to 38uM. (Only 28years too late, in 2004….DV). Genetic analysis showed R125W/T191M mutations in the CBS gene and homozygosity for the MTHFR C677T mutation. The first pregnancy occurred at 33years of age. At the 10th week of gestation, ultrasound showed a minimal detachment area, and rest was prescribed, without further events. Pyridoxine treatment was reduced to 300mg/day and acetylsalicylic acid (100mg/day) was added, while cystine and betaine treatments were maintained at the same doses.	180uM (cystine undetect-able) 56uM (cystine 38uM)	VitB6 300mg/d VitB6 300mg/d, Folate 10mg/day, Betaine 6g/day, Cystine 0.5mg/day
		Increased ingestion of milk derivatives to prevent osteopenia resulted in raised tHcy (100uM) and Met (75uM) levels, which suggested the need to start a Met-restricted diet (XMET Maxamaid, 75mg/day) at the 29th week of gestation. This resulted in a marked decrease in tHcy (10uM) and Met (27uM) levels. Spontaneous eutocic delivery (3.6weeks of gestation) resulted in a normal girl (birth weight 2840g, size 46cm, cephalic circumference 34cm, Apgar 9/10) with transitory jaundice. The postpartum period was uneventful in the mother; acetylsalicylic acid was withdrawn before delivery and heparin (20mg s.c./day) was added for 40days. The Met-restricted diet (tHcy 14uM, Met 27uM) and specific treatment for homocystinuria were continued. Following an early miscarriage (normal fetus), the patient became pregnant again at the age of 38. After an uncomplicated pregnancy, following the same treatment (pyridoxine, acetylsalicylic acid, folate, cystine, betaine, Met-restricted diet), with good metabolic control (tHcy 13uM, Met 35uM), a normal boy (Birth weight 3055g, Apgar 9/10) was born at 36.4weeks of gestation. Both children had normal development and attend regular school at present.	100uM 10uM 14uM 13uM	Changes as per text + milk derivativs Then + Low-Met diet ditto ditto
Orendac M et al (2004); Hum Mutat; Various Polish & Czech institutions	6 CBS--, VitB6-non-responsiv, Polish ancestry	From 5 unrelated families of Polish ancestry. All strongly VitB-nonresponsive	(pre-treatment) tHcy	CBS--Mutations
	#1	8y yes no? no yes 54 yes yes yes	283uM	C429G A1224-2C
	#2	12y yes yes no no 72 yes yes yes	385uM	C429G A1224-2C
Stroke	#3	5y yes yes yes yes 54 yes no yes	419uM	C684A C684A
	#4	5y yes yes yes yes 75 no no yes	385uM	T833C A1224-2C
Stroke	#5	3y yes yes yes yes 54 no no no	348uM	G442A G442A
	#6	9y yes yes yes yes 54 no no yes Where: AgeD = age at diagnosis, EctLent = Ectopia Lentis, Marfan = Marfanoid habitus, Osteopor = osteoporosis, Kyphoscol = kyphoscoliosis, IQ = IQ, Spast = spastic paresis Pyram = pyramidal signs, BA = brain atrophy	340uM	G1039+1T G1039+1T

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
Miles EW & Kraus JP (2004); J Biol Chem; NIH, Bethesda, Maryland, U Colorado, Denver, Colorado, both USA.	CBS--: Biochem: function, regulation	"Alternate Substrates and Reactions. CBS catalyses pyridoxal-phosphate(PLP)-dependent beta-replacement reactions (Eq2) in which the electronegative substituent (X) in the beta-position of the amino acid substrate is replaced by a nucleophile YH (cites reviews by Miles (1986) and Braunstein & Goryachenkova (1984)). Beta-replacement reactions (Eq2) are also catalysed by tryptophan synthase, O-acetylserine sulfhydrylase, and several other PLP enzymes. $XCH_2CH(NH_2)COOH + YH <-> XH + YCH_2CH(NH_2)COOH$ (Eq2) Where X is OH or SH and Y is S or S-alkyl. Amino acid substrates for CBS include L-serine (X is OH), L-cysteine (X is SH), 3-chloroalanine (X is Cl), and serine-O-sulfate (X is SO_4); Nucleophile substrates (YH) include L-Hcy, 2-mercaptoethanol, and H_2S….. Recent studies provide evidence that H_2S is a gaseous neuromodulator and smooth muscle relaxant and that H_2S is produced by CBS (cites Kimura 2002). Although the author suggests that H_2S is produced by a beta-elimination reaction with L-cysteine, H_2S may be a product of the beta-replacement reaction of L-cysteine with another thiol (cites Maclean and Kraus 2004). CBS will also very efficiently catalyze the formation of L-cysteine from L-serine and H_2S. This serine sulfhydrylase reaction may be an alternative method of cysteine synthesis and H_2S detoxification…... Regulation of Cystathionine Beta-Synthase. The human CBS gene is transcriptionally regulated by two promoter regions designated -1a and -1b. The major promoter (-1b) is serum and fibroblast growth factor-responsive and is down-regulated by insulin, growth arrest due to contact inhibition, nutrient depletion, or the induction of differentiation (cites Maclean et al 2002). The CBS -1b promoter is regulated in a redox-sensitive fashion by synergistic interactions between Sp1 and NF-Y and Sp1 and Sp3. Sp1 and Sp3 are the specificity factors 1 and 3, respectively, whereas NF-Y is the nuclear factor Y, a histone-like CCAAT-binding trimer. The dominant and indispensable role of Sp1 in regulating both GC-rich CBS promotors may allow tissue-specific repression by Kruppel-like factors. S1-like proteins and Kruppel-like factors are highly related redox-sensitive zinc-finger proteins that are important components of the eukaryotic cellular transcriptional machinery. In contrast to the relatively slow transcriptional response, AdoMet can instantaneously activate human CBS. Proteolytic removal of the C-terminal region also activates human CBS; the extent of the activation is similar to that observed with AdoMet….. AdoMet does not activate the yeast enzyme….. The role of heme in human CBS is still not clear. Heme is not essential for catalysis because it is absent in yeast CBS and T.cruzi CBS and because heme-free human CBS has activity. Human CBS may be regulated by the redox state of the heme. Whereas one group observed an ~2-fold decrease in CBS upon reduction of heme, another group did not observe this change." Note: No mention of metal enzyme cofactors…..
Schwahn BC et al (2004); Metabolism ; Montreal Children's Hospital, & associated institutions	CBS-+ mice	(Abstract) "We studied Hcy metabolism in mice with mild hyperhomocysteinemia due to CBS-+. Mice were fed diets (text: "This control diet….but contained choline at 5 instead of 10mmol/kg diet…diet thus contained only 20% less labile methyl groups than the AIN-93M reference diet, and similar diets have been considered to be methyl-sufficient in other dietary studies in rodents. The lower choline concentration was chosen to avoid saturating conditions for the choline-dependant remethylation pathway") supplemented with betaine or dimethylsulfonioacetate (DMSA); betaine and DMSA provide methyl groups for an alternate pathway of Hcy metabolism, remethylation by betaine-Hcy methyltransferase (BHMT). On control diets, CBS-+ mice had 50% higher tHcy than did CBS++ mice. Betaine and DMSA had similar effects in both genotype groups: liver betaine increased dramatically, while plasma Hcy decreased 40-50%. With increasing betaine supplementation, Hcy decreased by 75%. Plasma Hcy and BHMT activity both showed a strong negative correlation with liver betaine."

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
Ekinci B et al (2004); Movement Disorders; Cerrahpasa Medical School, Istanbul U, Turkey.	2 CBS--siblings	Patient 1. A 38year-old woman with generalized dystonia was followed-for 4years by our movement disorders clinic. She was born at full term after an unremarkable pregnancy, and when she was 7months old she experienced 3 febrile convulsions. She reached developmental milestones with delay such as walking at 1.5years and talking at the age of 3years; as a esult of mental retardation she could not attend primary school. Her family noticed a forward-bent spine when she was 2years old. She was diagnosed with homocystinuria at trhe age of 9years following the development of a cataract in the right eye. When she was 14, she developed involuntary tremor-like movementsand twisting contractions appearing first on the right foot and later on the left, which in time spread to the upper extremities. Since that time, axial dystonia has been gradually developing leading by age 20 to scoliosis with deviation of the body and neck to the right side. Later her hair became light-clored and she had a major depressive illness that responded well to treatment. In the late of her 20s she finally became wheelchair-bound and mostly dependent on others in her activities of daily living as a result of generalized dystonia…. Neurological examination of the patient at age 34 showed moderate mental retardation. Generalized dystonia was prominent on the right side leading to the right latero- and retrocollis, extensor posture in the rigtht leg and plantar flexion posture of the feet associated with a high amplitude dystonic tremor at the limbs as well as the head and trunk, causing twisting movement to the right and back of her body, worse with action. The physical examination revealed grey hair, bilateral pes cavus and equinovarus deformities and kyphoscoliosis. Ophthalmologic examination disclosed amaurosis in the right eye with corneal calcification and shrinkage of the iris and -10 dioptri myopia on the left eye. At the age of 38, the clinical picture became worse with gait and stance problems due to extensor right leg, inversion posturing of the right foot and plantar flexion of the feet and toes. Her speech became incomprehensible because of dystonic contractions of the maseter and laryngeal muscles. Achilles clonus in the right foot was present, but neurological examination was otherwise normal. Lab investigations of blood and urine, including the serum levels of copper and ceruloplasmin, VitB12, folate as well as 24hour urinary copper, the panel of liver and renal function tests were all normal except to an increased level of Hcy (I think they meant Hcy(ine), considering their normal value, and everything else….DV) in the urine at 85uM (Normal 0uM). MRI of the brain in T1-weighted images showed bilateral hyperintense gliotic lesions in the centrum semiovale, corona radiata and periventricular deep white matter. Axial T2-weighted images of MRI of the brain showed no abnormaility in the basal ganglia. The defined lesions were considered to be consistent with homocystinuria. L-dopa given for treatment of the dystonia (500mg/day) was ineffective. Repeated injections of botulinum toxin-A into the cervical and masseter muscles gave clinical benefit. Since the diagnosis of homocystinuria was established, the patient has taken pyridoxine 750mg/day, dipyridamole 225mg/day, folic acid 5mg/day and betaine 30mg/day. Patient 2. The first patient's 34year-old brother, an office worker, was diagnosed with homocystinuria at the age of 5years upon the development of a cataract. He was able to graduate from primary school but then developed stenotic valvular disorder of the aorta, varicose veins on the legs, hypertension and osteoporosis when he was age 10. 2years later, he had stiffness without pain in the back musculature and could not bend forward, but did not ferer to a physician. He was diagnosed as Karteneger's syndrome at the age of 27. In 1997, when he was 29, his score on the Short Test of Mental Status was 14 (normal = 38). In January 2002 his neurological examination revealed mild bradymimia, decreased eye blinking, bilateral bradykinesia, and rigidity which was prominent on the left side in the axial musculature in addition to dementia. He did not display dystonia. He was independent in his activities of daily living. We were recently informed that he had died of pneumonia in July 2002." Note: Some combination of VitB6-nonresponsivity, parental neglect, treatment non-compliance, late-ish diagnosis, and failure to include cystine supplementation in the treatment prescription, nor low-Met diet, must be considered in the etiology of very unsatisfactory and substandard outcomes for these two cases. Note also the lack(?) of Ectopia Lentis in either case.

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
Lebowitz EA (2004); J Vasc Interv Radiol; Santa Clara Valley Medical Center, San Jose, California, USA.	1 CBS--case	"A 19year-old man presented with gangrene of the right great toe that progressed to cyanosis of the distal half of the right foot within 2weeks. Risk factors noted initially included minimal smoking history, no diabetes mellitus, no hypertension, and no trauma, although the patient was an avid soccer player. Subsequent questioning during the following year revealed a history of trauma to the right knee with a baseball bat 6months before presentation and use of cocaine. Physical examination revealed a well-developed, well-nourished, athletic-appearing man with dry gangrene at the tip of the right great toe and wet gangrene at the base of the right great, second, and third toes. He was entirely nondysmorphic. All pulses were 4+, with the exception of the right popliteal, dorsalis pedis, and posterior tibial pulses, which were absent. An ankle-brachial index of 0.47 was measured in the right lower extremity and a non-invasive diagnosis of right superficial femoral artery occlusion was made. Treatment was started immediately with 325mg/day aspirin and 75 mg/day clopidogrel bisulfate and the patient was advised to quit smoking……. Initial Management. The patient was treated with tissue plasminogen activator at 1mg/hour through a Gragg-Macnamara valved infusion catheter positioned in the right popliteal thrombus and heparin at 200U/hour. No improvement was present the next day…..stopped, and the patient was brought to the operating room, where a right popliteal thrombectomy, saphenous vein patch angioplasty, and proximal popliteal vein-to-peroneal saphenous vein interposition grafting were performed. At operation, the patient had no atherosclerotic arterial disease, which suggested an etiology of thromboembolism from an unknown source…. The following laboratory data were obtained:….platelet count of 277,000/uL, prothrombin time of 11.7s,….antithrombin III activity of 82%, Protein C activity of 99%, Protein S activity of 101%, FVLeiden negative, lipoprotein(a) level <7mg/dL, VitB12 level 271pg/mL, echocardiograpy results demonstrating no embolic source……..MTHFR homozygous wildtype, tHcy 194uM, and cystathionine-beta-synthase (CBS) activity in fibroblast tissue of 0.0nmole/hour/mg (normal 5.4-18.5). Later Management. The patient was prescribed a regimen of anticoagulant therapy but presented again 1month later with exacerbated right lower-extremity ischemic symptoms. An arteriogram demonstrated occlusion of the right popliteal artery and graft. The patient underwent an operative thrombectomy and a saphenous vein bypass from his right superficial femoral artery to his peroneal artery. However, occlusion of the graft and occlusion of the distal right superficial femoral artery occurred in the ensuing 4months and required repeat thrombolysis attempts, operative thrombectomy, and a new operative bypass from the proximal superficial femoral artery to the posterior tibial artery with the use of a CryoVein because his native saphenous vein had already been used. At the time of the CryoVein bypass the diagnosis of homocystinuria was made (Note: It seems peculiarly obscure exactly when the diagnostic lab tests noted above in Initial Management were made, and why this diagnosis occurred so late in the course of events…DV) and medical therapy with VitB6, VitB9, and VitB12 was added to the warfarin, aspirin, and clopidogrel he was already taking. The tHcy decreased from 194uM to 11.1uM within 1month of treatment (Note: This is an unexpectedly great decrease in tHcy, given the CBS enzyme activity of nil, notwithstanding no report of any trialing of VitB6 addition to the enzyme, and this suggests VitB6-responsive CBS--, which is also consistent with the lack of any of the other stigmata of CBS--, which are more severe in untreated VitB6-nonresponsive cases – given that until this response was established he was suspect for non- or low-responsiveness, it would have been better to have also included cystine supplementation in the treatment, particularly as the other vitamin treatments re-route Hcy metabolism away from the CBS pathway that provides the sulfate potentially important in heparin sulfation which is potentially important in reducing thrombogenesis.... DV). When the CryoVein bypass graft thrombosed 2weeks after insertion, the patient was offered a below-the-knee amputation, which he refused. Consequently, repeat thrombectomy was performed and 2weeks later transmetatarsal amputation was performed. The wound healed well. Anticoagulation and vitamin treatment continue to the present, and in the 1.5years since the last thrombectomy, the graft has remained open and no new thromboembolic events have occurred… Discussion. The differential diagnosis for distal lower-extremity ischemia in a young patient includes……Homocystinuria was diagnosed and treated several months after presentation….. The principal lesson.is that interventional radiologists and surgeons may be called on to treat young patients with ischemic disease secondary to homocystinuria before the diagnosis has been made. The diagnosis may not even be considered because typical phenotypic features are absent. Such patients will fail to respond to standard therapies for peripheral vascular occlusive disease until their plasma Hcy levels are reduced, and the consequences may be severe.

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
De Luca M Casique L (2004); Mol Genet Metab; Center for biosciences and molecular medicine, Caracas, Venezuela	7 CBS--, Venezuel-an	"This center has detected (no timespan provided. DV) 53 suspected cases out of 24,755 sick or healthy children, using the cyanide-nitroprusside technique in urine (no basis for the application thereof provided. DV). In order to confirm the homocystinuria diagnosis by quantifying tHcy, 26 out of 53 families have been contacted until the present, and 9 cases were confirmed including 2 couples of siblings, though no DNA samples of 2 of them were available. These 9 patients were diagnosed on the basis of a severe hyperhomocysteinemia in combination with clinical manifestations of CBS--. 10patients out of the total of the confirmed 26 have already died (no mention of the other 26 – 9 – 10 = 7. DV)…… Blood samples were obtained from 7 Venezuelan HCU patients, including 2 sib pairs and 5 unrelated genotypes….. Patients were not compliant with dietary therapy….. They presented the total number of cases studied (thusly. DV) so far in Venuezuela."		
	CBS--#	AgeD AgeO Ethnic Mut1 Mut2 MTHFR Skel Eye CNS VitB6resp	tHcy	Treat
	#930	4y 10m Venez C699T C699T hetz yes yes yes partial	211uM	yes
	#1110, sib	10y 2y Venez C699T C699T norm yes yes yes partial	209uM	yes
	#2255	19y 17y Portug C1080T C1080T norm no yes ?	161uM	no
	#MB, sib	21y 7y Portug C1080T C1080T norm no yes yes	126uM	no
	#2954	4y 2y Indigen +Venez T191M T191M hetz no yes yes partial	193uM	yes
	#3800	11y 2y Venez D234N D234N hetz yes yes no	150uM	yes
	#5169	7y 2y Venez Q243X C699T norm yes yes yes	261uM	yes
		Where: AgeD = age at diagnosis, AgeO = age at onset, Ethnic = ethnic origins, where indigen = indigenous, Venez = indigenous + Spanish/Italian, and Portug = Portuguese, Mut1,Mut2 = mutations on alleles 1 & 2, MTHFR = MTHFR C677T mutation where norm = homozygous normal, hetz = heterozygous, Skel = Marfanoid habitus/ scoliosis/ skeletal abnormalities, Eye = Ectopia Lentis/ glaucoma/ severe myopia, CNS = psychomotor retardation/ mental retardation/ abnormal electroencephalogram/ seizures/ psychosis, VitB6resp = VitB6-responsivity where partial = partially responsive to VitB6, Treat = "Referred by the parents" whatever that means?..... And DNA mutations are given where provided by the authors, and amino acid substitutions provided in lieu where not. Note: It appears safe to assume that the tHcy was that at the time of diagnosis, ie pre-treatment (they may have been given VitB6 and complied with that, while all being noted for dietary non-compliance. Note: It is difficult to infer whether the clinical manifestations apply to the time of diagnosis, or to the present – information not provided.		

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
Moat SJ et al (2004); Hum Mutat; U Colorado, Colorado, USA, Royal Manchester Children's Hospital, Manchester UK, & others		(abstract) "The molecular basis of CBS-- has been studied in 536 patient alleles with 130 different mutations described. To date, no study has reported on the incidence of any of the reported mutations in patients from the UK and the US. We developed a new antisense oligonucleotide (ASO) PCR/hybridization method to screen for 12 of the most frequent CBS mutations in 14 unrelated patients from the UK and 38 unrelated patients from the US, a total of 104 independent alleles. We determined 16/28 (57%) and 28/76 (37%) of the affected alleles in the UK and US patients, respectively. 4 different mutations were identified in the UK patients (c.374G>A, R125Q; c.430G>A, E144K; c.833C>T, I278T; c.919G>A, G307S) and 8 mutations identified in the patients from the US (c.341C>T, A114V; c.374G>A, R125Q; c.785C>T, T262M; c.797G>A, R266K; c.833C>T, I278T; c.919G>A, G307S; g13217A>C(del ex 12); c.1330G>A, D444N). The I278T was the predominant mutation in both populations, present in 8 (29%) of independent alleles from the UK and in 14 (18%) of 76 independent alleles form the US. The incidence of the G307S mutation was 21% in the UK patients and 8% in the US patients. The spectrum of mutations observed in the patients from the UK and US is closer to that which is observed in Northern Europe and bears less resemblance to that observed in Ireland." "The G307S mutation has been detected in patients from the US and Australia with "Celtic" origin including families with Scottish, English, French, and Portuguese ancestry. However, the G307S mutation has not been detected in tested alleles in Italy, the Netherlands, Germany, Spain and the Czech Republic. Interestingly this mutation was recently detected in Norway, indicating that this allele may have originated in Scandinavia and spread elsewhere [Kim et al., 1997]. The I278T mutation is panethnic…..Three mutations G116R, I152M, and C165Y were not detected in the 104 alleles. Most surprising is the absence of the C165Y mutation found previously in 8 alleles in patients of Dutch, Czech and South African origin."
Linnebank M et al (2004); Hum Mutat; U Hospital Bonn, Bonn, Germany, U Hospital Muenster, Muenster, Germany, Inst of Inhertited Metabolic Disorders, Prague, Czech Republic, U Colorado, Colorado, USA.	17 CBS-- patients of Caucasian origin carrying the mutation c.1224-2A>C.	"17 CBS-- patients of Caucasian origin carrying the mutation c.1224-2A>C were included in this study. They were known from the CBS database, from personal communication or from our own hospitals and laboratories. Except for 1 patient of Turkish origin, all these patients were of "Central" Europe ancestry: 4 Polish, 4 Slovak, 3 Czech, 2 German, 1 Austrian, 1 Croatian, 1 Hungarian….In the present study, response to VitB6 supplementation – usually 500-1000mg/day – was defined as a decrease of fasting tHcy to <=50uM, partial response as a decrease to 50-200uM…. The clinical spectrum varied from rather mild phenotypes with isolated thrombotic events or ocular lense dislocation to severe phenotypes with disabling mental retardation and generalised involvement of connective tissue. At the time of diagnosis, the 2 patients homozygous for c.1224-2A>C presented with severe phenotypes. They exhibited VitB6-nonresponsiveness, while VitB6-responsiveness of the other patients depended on the mutation of the second allele…. None of the patients with thrombotic events carried the FVLeiden or the prothrombin polymorphism confirming that these are not necessary for thrombotic events in CBS--…… In the 17 patients we analysed 10 polymorphisms in the CBS gene and constructed haplotypes by maximum likelihood approach. All 19 c.1224-2A>C chromosomes shared the same haplotype. These data indicate that c.1224-2A>C originated from a single mutational event, and a founder effect may be responsible for the origin of the mutation in Europe."

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
Refsum H et al (2004); J Pediatr; U Oxford, UK, U Bergen, Bergen, Norway, Rikhospit-alet U Hospital, Oslo, Norway.		"From February to April 1999, ~5000 samples were randomly selected among ~12,000 capillary blood samples that weee sent to the RUH, Oslo, for routine newborn screening of phenylketonuria and congenital hypothyroidism……..Genotyping for CBS mutations was performed in 1133 random samples. We have previously identified 6 different mutations among Norwegian families CBS--: 785C>T, 797G>A, 833T>C, 919G>A, 959T>C, and 1105C>T. Using a multiplex, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry method (cites Harksen A et al 1999). The prevalence of homocystinuria was calculated on the basis of the assumption that Hardy-Weinberg equilibrium exists and that babies with 2 mutated alleles will have homocystinuria. When the frequencies are "w" for the wild-type allele and "m" for the mutant allele, the frequencies will be w2, 2wm, and m2 for the homozygous wild-type, the heterozygotes, and the homozygous mutant genotype, respectively. Since we know the prevalence of w2 (CBS++) and 2wm (CBS-+), we can calculate the prevalence of m2 (CBS--). Mutation VitB6-responsive n % 785C>T no 0 .00 919G>A no 2 0.18 959T>C no 0 .00 797G>A yes 1 .09 833T>C yes 7 0.62 1105C>T yes 18 1.59 Any Mut 28 2.47 ….Overall CBS-+ was observed in 2.47% (95%CI, 1.57-3.37) of the samples, yielding an estimated birth prevalence of CBS-- of ~ 1:6400 (I agree DV)…. We limited our investigation to 6 CBS mutations previously found in Norwegians, but there are numerous other CBS mutations associated with homocystinuria. Thus our estimate is probably too low….. However, we do not know whether CBS mutations in the mother or fetus cause reduced reproductive fitness. This could lead to a lower prevalence of live-born babies with CBS--. Hence the the H-W equilibrium may not exist. Another factor is clinical penetrance, that is, whether homozygosity or compound heterozygosity for any pair of these mutations will lead to a clinically evident homocystinuria (means other CBS-- stigmata? DV). We found that 90% of the mutated alleles in newborn infants are associated with a pyridoxine responsive phenotype. This proportion differs markedly from the widespread experience that only ~50% of patients respond to pyridoxine. Pyridoxine-responsiveness is usually associated with a less severe clinical disease. The discrepancy between the genetic findings in newborn blood samples and that observed in patients could suggest that many CBS-- have a pyridoxine-responsive variant with mild or no symptoms. Another possibility is that diagnosis is frequently missed because the medical community is not fully aware of this disorder and its clinical manifestations. Our findings indicate that novel high throughput techniques for mutation detection may be a useful procedure to identify babies with mutations causing both mild and severe variants of CBS--. However this approach will miss cases caused by unknown or rare CBS mutations and may include those with a genetic defect but normal biochemical and clinical phenotype. Thus, the optimal approach for detection of CBS-- remains to be determined."

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease	Hcy level	Treatmnt and biochem etc contexts
Mulvihill A et al (2004); J AAPOS; National Centre for Inherited Metabolic Disorders, The Children's Hospital, Dublin, Ireland.	27 CBS--, Irish, 70% VitB6-non-resp G307S	"Subjects. All patients attending the Irish NCIMD were considered for inclusion in the study. The patients were either diagnosed through the national newborn screening program or clinically detected. The initial findings of hypermethionemia and hyperhomocyateinemia were further confirmed by either enzymology of CBS in fibroblast or CBS mutational analysis. Over 70% of the defective alleles in this study cohort were due to 919G>A (G307S). Treatment and Metabolic Control. ….a trial of pyridoxine is given.to ascertain in vivo response of the patient to pyridoxine while remaining on a normal diet. Half of the patients with Hcy-uria worldwide are pyridoxine responsive, which makes pyridoxine the mainstay of treatment in these patients. In pyridoxine nonresponsive patients, dietary treatment with methionine restriction and cystine supplementation is commenced. In addition, pyridoxine and folic acid with VitB12 are also prescribed (Should give some quantitative info on trends in prevalence on the use of the addition fo folate and VitB12, and to which patients it is applied (ie as function of compliance with dietary recommendations), and also, given their exact wording here, whether cystine supplementation is also included with any treatments using pyridoxine, folate and VitB12. DV). For this study cohort of patients, treatment would have started within 6weeks of birth for those detected through newborn screening and at the time of diagnosis for the late detected patients. Biochemical control was monitored using free Hcy(ine) ("fHcy(ine)" DV). Free Hcy is normally undetectable. Each patient studied would have had at least 8 measurements of fHcy per year."		Treatment (see column 2 to left just above here)
	Group#,n	OcularPathology Age AgeD(mean:individuals) fHcy(ine)	tHcy (derived fromn fHcy)	Complian-ce
	I, n = 14	None 21.4y :12*NBS, 2.5y, 15y <11uM RefractiveError -0.25D AxialLength 23.4mm+/-0.9	<110uM	Good
	II, n = 6	Phacodonesis/EctLentis 26.1y :3*NBS, 1.5y, 2.4y, 27y >11uM RefractiveError -10.7D AxialLength 23.8mm+/-1.8	>110uM	Poor (derived fr Hcy level)
	III, n = 7	1or2 EctLent into vitrous 27.5 y 8.8y: 2.8, 3.0, 4.1, 5.3, 7.2, 11.5, 28y RefractiveError +12.9D AxialLength 24.9mm+/-0.9**	>200uM roughly, assumed	NotApplic
		Where: ss for RefractiveError was not provided, and ss (**) for AxialLength was vs Group I, P = .0018, Age = mean age at time of study, AgeD = age at time of diagnosis, NBS = newborn screening, EctLentis, EctLent = Ectopia Lentis, And, where tHcy was derived using the relationship of fHcy(ine) (data values provided by Mulvihill et al here) to tHcy reported by Bonham et al (1997), such that: tHcy = 60 + 4.5(fHcy(ine)) for values of fHcy(ine) < 20uM, and tHcy = 60 + 90 + (fHcy(ine) – 20) for values of tHcy(ine) >=21uM		

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal,	Study Group	Disease
Gomber S et al (2004); Indian Pediatrics; Guru Teg Bahadur Hospital, Delhi, India	1 CBS--	An 8y-old boy…..first presented with pallor……history of progressively increasing pallor for the past 2years and of receiving one blood transfusion at the age of 6years…. no: pedal edema, jaundice, petechia, ecchymosis, hepatosplenomegaly, lymphadenopathy, sternal tenderness, Hb 7.15g/dL, total leukocytes 5700/mm^3, normal differential count, platelets 80,000/ mm^3, reticulocytes 1.8%. Peripheral smear revealed normochromic RBCs with few macrocytes and reduced platelets. Mean corpuscular volume 128fL, mean corpuscular Hb 37pg. Bone marrow megaloblastic changes. Patient started on treatment for megaloblastic anemia: folate 5mg/day + VitB12 100ug/day oral. After 1month of treatment the Hb improved to 10.4g/dL and mean corpuscular voume reduced to 98fL while the reticulocyte count increased to 4%. After initial improvement in anemia the patient was lost to follow-up. After 2years the patient again came with pallor, and redness and pain in the right eye. On examination the intraocular pressure of the right eye was increased and bilateral inferonasal subluxation of the lens was found. The right lens showed cataract and atrophic patches on the iris while the left lens was clear. The blood pressure was within normal limits. Hb 4.5g/dL, total leukocytes 7500/mm^3, platelets 69,000/ mm^3, Mean corpuscular volume 112fL, mean corpuscular Hb 37pg. Peripheral smear showed anisopoikilocytosis with macrocytes, ovalocytes, tear drop cells, polychromatic cells with course basophilic stippling, cabot rings and few microcytic hypochromic cells. **tHcy 72uM** (normal 5-15uM), VitB12 364pg/ml (normal 200-800pg/ml). Stool examination for fat malabsorption and reducing substance was negative. Normal urinalysis. Normal Doppler studies. No evidence of osteoporosis in radiographs of long bones… Diagnosed CBS-- and started on VitB6 200mg/day + folic acid 5mg/day. "There was a disappearance of fasting plasma methionine and urinary homocysteine and methionine after 8weeks of treatment." After 12wks treatment, Hb 13.3g/dL, mean corpuscular volume 85fL, mean corpusc'r Hb 28pg. Note: Do the relatively low untreated tHcy levels (reduction to this level by (various) treatments generally (universally?) prevents ectopia lentis) here with the finding of Ectopia Lentis in the apparent presence of folate deficiency indicate an interaction between CBS--'s metabolic derangements and folate deficiency? That is, unrelated to low folate increasing Hcy levels?....
Bar-Or D et al (2004); Biochem Biophysic Res Comm; Swedish Medical Center, Englewood DMI BioSciences Inc, Englewood, U Colorado, Denver, all of Colorado, USA, Bowman Research Ltd, Newport, Gwent, UK	CBS-- mice, and CBS++ rats	"In oxidising environments (they state that this is the predominant environment in blood. DV), cysteine (Cys) is oxidized into its cystine disulfide which interacts with protein-free sulfhydryl groups, such as albumin Cys34, to form cysteinylated species of the protein. Cys34 is the only free sulfhydryl group on albumin, which accounts for over 80% of the total free thiols in plasma (cite Carballal S 2003). In cases of ischemia, when hypoxemia and acidosis are present, the activity of CBS in the area of ischemia is decreased (cite Taoka S 1998) leading to the accumulation of Hcy. When equilibrated with the normal non-ischemic circulation during reperfusion, Hcy is metabolized by non-inhibited CBS from non-ischemic areas into Cys. Consequently, Cys id oxidized into cystine which reacts with free sulfhydryl groups on proteins. Albumin Cys 34 is a major reactant antioxidant "buffer" molecule which absorbs the oxidant cystine to form a cysteinylated albumin species (CysAlb) and a free Cys (cite Carballal S 2003)." "Abstract. ….Rats….The 3 treatment groups were as follows: Midline abdominal incision (Group A, n = 10); Ditto and followed by ligation of the superior mesenteric artery for 2hours (Group B, n = 3); Ditto followed by reperfusion for 1hour (Group C, n = 10). Hcy levels were 2.5-fold higher in Group C than Group A (P<.05), Hcy levels were 1.9-fold higher in Group C than Group B (P<.05). In Group A 51% of albumin were modified into CysAlb; In Group B 73% of albumin were modified into CysAlb; In Group C 100% of albumin were modified into CysAlb. …Mice…. CBS-- mice: tHcy 180uM; %CysAlb 0%; %HcyAlb 23%; Normal mice: tHcy 6uM; %CysAlb 24%; %HcyAlb 0%" "More importantly, the modification of 100% of albumin into cysteinylated species (as seen in Group C) indicates the loss of the majority of oxidant buffering capacity in the extracellular environment (no citation given – in humans, could vary widely as a function of dietary or supplemental (or pre-op IV….) antioxidant intake. DV)." Note: In mice, the loss of albumin's Cys-oxidant-buffering capacity is approx equal in CBS--/++.

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
Robert K et al (2005a); The Anatomical Record Part A; U Paris, INSERM, & U Necker Enfants Malades, All of Paris, France.	CBS--, CBS-+, CBS++ mice	"At weaning, CBS--, CBS-+, and wild-type CBS++ mice from the same litter were fed on a diet supplemented with 1.6g/kg choline chloride salt because CBS—mice died young when fed a standard laboratory diet. CBS-+ and CBS++ littermates were also fed the same supplemented chow to avoid differences due to diet…..CBS--/-+/++ mice were identified by PCR… We measured plasma tHcy in 3-8month-old mice. tHcy was 50-fold higher in CBS-- mice than in CBS++ mice (205uM+/-86 vs 3.9uM+/-0.9; P <.0001; n = 4 each)…..CBS-+ mice...tHcy was 2-fold higher than in CBS++ mice (9.1uM+/-2.4 vs 3.9uM+/-0.9; P<.0001; n = 4 each)….. Body weight mean of 3, 5, 8months Kyphotic Angle CBS-- 21.2g+/-1.1 P<.007 vs CBS++ 105+/-7 P<.004 vs CBS++ CBS-+ 25.0g+/-1.1 nss vs CBS++ 120+/-10 P<.005 vs CBS++ CBS++ 27.2g+/-1.1 141+/-4 Lengths(mm) @1month: Femur Tibia Humerus Ulna CBS-- 8.0+/-0.3[a] 10.0+/-0.1[b] 7.6+/-0.1[c] 9.0+/-0.1[d] CBS-+ 13.5+/-0.3 15.7+/-0.2 10.6+/-0.3 12.4+/-0.1 CBS++ 14.4+/-0.2 16.0+/-0.5 10.8+/-0.4 12.1+/-0.1 Lengths(mm) @3months: Femur Tibia Humerus Ulna CBS-- 15.0+/-0.1[e] 17.2+/-0.1[f] 12.5+/-.07 13.6+/-0.1 CBS-+ 14.5+/-0.2$_e$ 16.9+/-0.2[g] 11.5+/-0.2 13.3+/-0.1 CBS++ 16.0+/-.04 17.9+/-0.2 12.1+/-0.2 13.7+/-0.1 Where: a is P<.0005; b is P<.008; c is P<.002 +; d is P<.0001; all vs CBS++; e is P<.0005; f is P<.007; g is P<.01 +; all vs CBS++; (But, note: The reduction of variances in the last group of data is the reason for the ss values being similar to those of the foregoing group of data – this may be coincidental….DV) …..Microscopic examination showed expansion of the cartilaginous growth plate with the disruption of the ordered arrangement of chondrocytes during differentiation in CBS-- mice, especially in the hypertrophic zone. Cartilage differentiation of CBS-- mice was delayed at 1month compared to age-matched CBS++ mice, and hence the growth of the long bones was disturbed…… Therefore, hyperhomocystenemia due to CBS-- in mice was associated with delayed ossification rather than with failed ossification….. Growth retardation is not a characteristic of human patients with CBS--. Nevertheless, it has also been observed in rats fed excessive methionine or homocystine (cites Cohen et al 1958)…. Some of the skeletal abnormalities, such as kyphosis and arachnodactyly observed in CBS-- mice, shared an outward resemblance to those observed in mice with a hypomorphic fibrillin-1 mutation, which leads to Marfan syndrome-like manifestations (cites Periera et al 1999)….. Morover, CBS is not ubiquitously expressed in in mouse tissues (cites Robert et al 2003). In the early stages of development, the gene is expressed only in liver and in the skeletal, cardiac, and nervous systems. In the skeletal system, expression can be detected at early stages in chondroblasts in areas at which bone ossification is believed to be initiated. In adult mice, the signal for enzyme synthesis persists in the perichondrium in cartilage and in the periosteum in bone. This indicates that dietary cysteine is insufficient in some organs and there is a requirement for the local production of cysteine or its metabolic byproducts by transsulfuration. It should be noted that MTHFR-- mice, which do not have an impaired transsulfuration pathway, are significantly lighter and develop more slowly than MTHFR++, but kyphosis is only observed occasionally (cites Chen et al 2001). Although CBS-- phenotype appears to be caused by dramatically elevated Hcy, it is possible that blocking cysteine biosynthesis could contribute to the skeletal defects observed in CBS-- mice, where the transsulfuration pathway is impaired."

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
Robert K et al (2005b); The Anatomical Record Part A; U Paris, INSERM, & U Necker Enfants Malades, All of Paris, France.	CBS--, CBS-+, CBS++ mice???	(Same group of mice as for their 2005a paper immediately above) "At weaning, CBS--, CBS-+, and wild-type CBS++ mice from the same litter were fed on a diet supplemented with 1.6g/kg choline chloride salt because CBS—mice died young when fed a standard laboratory diet. CBS-+ and CBS++ littermates were also fed the same supplemented chow to avoid differences due to diet…..CBS--/-+/++ mice were identified by PCR… We measured plasma tHcy in 3-8month-old mice. tHcy was 50-fold higher in CBS-- mice than in CBS++ mice (205uM+/-86 vs 3.9uM+/-0.9; P <.0001; n = 4 each)…..CBS-+ mice...tHcy was 2-fold higher than in CBS++ mice (9.1uM+/-2.4 vs 3.9uM+/-0.9; P<.0001; n = 4 each)….. (Abstract) Background & Aims. CBS-- causes severe hyperhomocysteinemia, which confers diverse clinical manifestations, notably liver disease…… Methods. The degree of liver injury and inflammation was assessed by histologic examination, by measurements of products of lipid peroxidation, and by formation of carbonyl groups on protein as a measure for the occurrence of protein oxidation. Analysis of profibrogenic, proinflammatory factors and cell apoptosis was performed by Western blots, real-time quantitative PCR, caspase-3 activity, DNA laddering, and TUNEL assay. Results. Histologic evaluation of liver specimens of 8- to32-week-old CBS-- mice showed that CBS--mice develop inflammation, fibrosis, and hepatic steatosis, concomitant with an enhanced expression of tissue inhibitor of metalloproteinase-1, alpha-smooth muscle actin, pro(alpha)1 collagen type 1, transforming growth factor-beta1, and proinflammatory cytokines. Moreover, even if the proapoptotic protein Bax was dominantly expressed and Bcl-2 was down-regulated, caspase-3 was not activated, DNA laddering was not detected, and number of positive TUNEL cells was not increased in liver of CBS-- mice compared with CBS++ mice. Conclusions. The results show that hyperhomocysteinemia in liver of CBS-- mice promotes oxidative stress, which may cause mitochondrial damage in association with activation of hepatic stellate cells, leading to liver injury. The absence of caspase-3 activation, DNA fragmentation, and TUNEL-positive cells shows that protective signals may counteract apoptotic signals in liver of CBS--mice….. …..Densitometric analysis of immunoblots demonstrated an approximate increase of 30% in oxidatively mocified proteins (P<.01; n = 4 in each group)….. The levels of MDA and 4-HNE in liver of 12week-old male CBS-- mice were ~30% higher than in CBS++ mice (29.7+/-3.2 vs 21.4+/-1.5 pmol/mg protein respectively) (P<.02; n = 5 / group). Taken together these results demonstrate an enhanced protein oxidation and lipid peroxidation in liver of CBS—mice….. Table 2. Analysis of relative expression of TGF-beta1, alphaSMA, Pro-(alpha)collagen-1, TNF-alpha, IL-6, and CD14 Gene based on Q-PCR data obtained from CBS++ and CBS-- livers. mRNA CBS++ CBS-- ss TGF-beta1 1.01+/-0.1 8.8+/-2 P<.003 alphaSMA 1+/-.03 7.7+/-0.3 P<.0001 Pro-(alpha)collagen-1 1.06+/-0.2 9.19+/-.07 P<.0001 TNF-alpha 1.06+/-0.2 99.2+/-7 P<.0001 IL-6 1+/-.04 3.5+/-0.1 P<.0001 CD14 Gene 1.06+/-0.2 1.62+/-.04 P<.03 Discussion. Lipid peroxidation is the most important mechanism in the pathogenesis of acute liver damage….An inhibition of methyltransferases is considered to be one of the causes of fatty liver because the liver is the organ in which 85% of all transmethylation reactions occur. Choumenkovitch et al (2002) have shown an increase in SAH levels, a powerful inhibitor of of SAM-dependent transmethylation reactions, associated with lower genomicDNA methylation status in liver of CBS-- mice…. CBS-+ mice fed a hyperhomocysteinemic diet have increased hepatic cholesterol and triglyceride levels through increased hepatic expression of genes involved in cholesterol and triglyceride synthesis, uptake, and storage, which results in hepatic steatosis (cites Werstuck et al 2001)."

Cystathionine Beta Synthase Severe Homozygous (or compound heterozygous) Mutations

Authors, Journal, Site	Study Group	Disease
Riksen N et al (2005); Arterioscler Thromb Vasc Biol; U Medical Centre Nijmegen, Nijmegen, The Netherlands		"According to this hypothesis, a Hcy-induced fall in extracellular adenosine contributes to the cardiovascular sequelae of hyperhomocystenemia. Fundamental to this is the reversibility of the reaction in which SAH is hydrolysed to Hcy and Ado. Although the equilibrium constant of this reaction favors SAH synthesis, under physiological conditions SAH is hydrolysed to to Hcy and Ado, because both reaction products are rapidly metabolized. In hyperhomocysteinemia, the reaction shifts towards SAH synthesis at the expense of free intracellular adenosine. Subsequently, facilitated diffusion of extracellular adenosine into the cells through the dipyridamole-sensitive equilibrative nucleoside transporter is enhanced, limiting stimulation of membrane-bound adenosine receptors. By stimulation of these receptors, extracellular adenosine induces several effects, which could protect against the development of atherosclerosis and thrombosis and against ischemia-reperfusion injury (cites Riksen et al 2003, Rongen et al 1997). Particularly in situations of hypoxia or ischemia, when the concentration of adenosine increases rapidly, these effects (not noted DV) work in concert to protect the affected tissue….. Subjects. …adult patients with hyperHcy due to CBS-- from our outpatient clinic were asked to participate if their fasting tHcy was >20uM despite treatment. Exclusion criteria were mental retardation, previous vascular events, asthma, and oral anticoagulation. 12 patients were considered eligible and 9 participated. (tHcy = 93uM+/-25) Their Hcy-lowering therapy consisted of VitB6 250-750mg, n = 9, Folic acid 5mg, n = 9, VitB12 10ug/day orally or 1mg intramuscularly each 2months, n = 7, Betaine anhydricum 6g, n = 7, With further treatments being acetylsalicylic acid 80mg in 1 patient (stopped 1week before experiment), and alendronic acid 70mg/week in 2 patients. A control group of 8 healthy volunteers was composed with similar age and BMI as the patient group. (tHcy = 9uM+/-1) (Abstract) Methods and Results. Infusion of adenosine (0.5, 1.5, 5.0, and 15.0ug/min/dL forearm) into the brachial artery increased forearm blood flow, as measured with venous occlusion plethysmography, to 2.9+/-0.4, 4.3+/-0.5, 5.6+/-1.1, and 9.6ml/min/dL+/-2.1 in the patients and to 2.8+/-0.6, 4.4+/-1.0, 9.0+/-1.7, and 17.06ml/min/dL+/-3.1 in the controls (P<.05) However, adenosine-induced vasodilation in the presence of dipyridamole (100ug/min/dL) was similar in both groups (P = 0.9). Additionally, in isolated erythrocytes, adenosine uptake (from 1uM solution) was accelerated by incubation with Hcy (100uM, pre-incubation for 10minutes pre-adenosine addition) (half-time 6.4+/-0.3 versus 8.1+/-0.5minutes, P<.0001), associated with increased intracellular formation of SAH (P<.0001). Conclusions. In hyperhomocysteinemia, adenosine-induced vasodilation is impaired but is restored by dipyridamole. Accelerated cellular adenosine uptake probably accounts for these observations. These impaired actions of adenosine could well contribute to the cardiovascular complications of hyperhomocysteinemia."

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Peer/ Expert Review Section to follow below.

Peer and Expert Review

A fairly comprehensive effort was made to solicit comprehensive peer/ expert review of/for this book.

All those contacted were approached via an email including the following:
"The book has significant differences from your own work and that of others' in the field, though is muchly informed by that - accordingly a condition I will strictly adhere to is that your commentary would be reproduced in the final published form, in a section on peer review commentary, in the book exactly as you provide it, verbatim and in full, no matter how negative your comments - however, prior to the publication I have the right of reply (within 1 week), and then you and the others have the right of reply (within 1 week) to my replies, and so on until we have nothing new to contribute to the discussion - at whatever point you finalise your commentary and subsequent replies absolutely everything you have contributed would be included in the published book exactly as you provide it."

The following workers in the field of CBS-deficiency Homocystinuria, contacted by email, represent the large majority of more prominent treatment centres worldwide:

Professor Harvey S Mudd, Scientist Emeritus at the Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, the USA, and author of the relevant chapter in the most authoritative The Metabolic and Molecular Bases of Inherited Disease (Scrivner CR, Beaudet AL, Sly WA, et al, eds). Prof Mudd contributed the reviewing and suggestions reproduced verbatim below.

Professor Sufin Yap, a clinician, researcher and teacher of many years, at various times being a Consultant Paediatrician in Metabolic Medicine at Sheffield Children's Hospital, and similarly involved at the National Center for Inherited Metabolic Disorders, and the Society for Inherited Metabolic Disorders, mostly in Dublin of Ireland, The UK.
Prof. Yap was unable to review this book, but provided information as acknowledged in citation.

Professor Bridgett Wilcken, a clinician, researcher and teacher of many years, in paediatric and metabolic diseases, of the Children's Hospital Westmead, The University of Sydney, Australia.
Prof. Wilcken was initially approached around 2010 with a view to co-authorship of a paper I was attempting to get published in scientific journals, the paper being around the statistical and other re-analyses of the data and analyses of the Yap (2001) paper (of which she was a co-author) much as appears here in chapter 3. At that time she responded with a disputing of my analyses, to which I responded, inviting her further response on more than one occasion, which she declined to make. On deciding to publish as a book instead of in journal articles, in 2013 I notified her that I would be including our earlier dialogue on the matter in this book, as that matter was included much in its entirety in this book, and offered her the opportunity to provide me with a replacement reviewing of the whole book in its present form. On receiving as yet no response I proceed to reproduce the dialogue of 2010 verbatim below.

Professor GH Boers, Professor FJ Trijbels, Professor HJ Blom, and Dr. LA Kluijtmans, all of The Netherlands, I tried (after much failure elsewhere) to acquire the email contacts for from Prof Kozich of Czechoslovakia, but he informed me that Profs. Boers and Trijbels had retired quite some time ago, and that Prof. Blom and Dr. Kluijtmans are biochemists/ molecular geneticists, not clinicians, so I ceased

attempts to contact them, though I would welcome any feedback they would care to make and include it in the book soon after receipt.

Professor Viktor Kozich and colleagues, of Czechoslavakia were requested to review or comment, but other than a congratulation (probably merely routine politeness) from Prof. Kozich I have received nothing in response.

In Sweden, Associate Professor Olov Ekwall, Specialist Physician Annika Reims, Dietician Karina Eftring, all of The Queen Silvia Children's Hospital, Gothenburg; and Associate Professor Anna Nordenström, Specialist Physician Rolf Zetterström, and Dietician Carina Heidenborg of Children's Hospital, Karolinska University Hospital, Huddinge, Stockholm; and Associate Professor Ulrika von Döbeln, of the Centre for Inherited Metabolic Diseases (CMMS), Karolinska University Hospital, Solna, Stockholm, were all invited to contribute review or comments, but none have yet been recieved.

Response from Professor Harvey Mudd:

Thanks very much for your response Prof. Mudd.
I agree with virtually every change you suggested, and will carry them out as detailed here below.
I respond in detail to your comments one by one as you provided them, below.
What appears below will be reproduced exactly verbatim in the last section/ chapter of the book, so there should be no chance of the level of your involvement being misrepresented.
Any further commentary would be very welcome, and as I am self-publishing via Amazon's CreateSpace (Print on Demand), even if you comment after the initial publication, which is unlikely to be sooner than two weeks from now, I will be able to modify the book as printable in the future. Of course the earlier I can include the expert review / commentary in books printed or downloaded the better.
Thanks again,
David Vance

From: muddh@mail.nih.gov
To: davidjmvance@hotmail.com
Subject: RE: Thanks; CBS-- Homocystinuria book
Date: Sat, 16 Mar 2013 22:49:36 +0000

HM: I am writing hurriedly. Chiefly to respond to your request that I acknowledge receipt of your book – which I did receive. Clearly you have done a great deal of work and analysis – too much for me to go over in any detail in the few moments I have had available that were not distracted by other events. I offer here a few relatively minor suggestions for changes that occurred to me as I no more than skimmed your draft:

HM: p.8 , line 4: Mention might be made of disorders of homocysteine remethylation in addition to MTHFR deficiency that are not covered: cblC disorder (probably currently the most frequently detected cause of elevated tHcy when screened for), cblD, cblE and cblG disorders.

(DV: Yes, I will make this addition)

HM: p. 9, text line 2 (and elsewhere): Either replace “homocystinurics” with “CBS-deficient individuals” or explain that the word “homocystinuric” is used with a restricted meaning.

(DV: OK, I will change "homocystinurics due to cystathionine beta synthase deficiency" to "cystathionine beta synthase deficient homocystinurics")

HM: p. 19, first line of 3.3 and many other places: In most instances what is being discussed when you say “homozygous CBS-deficiency” is in reality either homozygous or compound heterozygous-CBS deficiency. Probably most of the headlines on p. 55-157 have similar mis-terminologies (although there may be a few papers where only homozygotes are discussed).

(DV: Yes, I will change to "homozygous or compound heterozygous CBS deficiency")

HM: p. 29, middle paragraph: I may have missed, but it would be a convenience for the reader if, when you first discuss “homocysteine” levels, you specify the difference between homocysteine and total Hcy (tHcy) and, perhaps, present examples of how to covert relevant values to tHcy.

(DV: Yes, I will put " 'total' homocysteine (tHcy) (homocysteine in Hcy-Hcy dimer + Hcy-Cysteine mixed disulfide + Hcy bound to plasma proteins + free homocysteine)"

At the bottom of Table 1 I have already detailed the algorithms I used to convert other Hcy values to tHcy)

HM: p. 55-157: For the many instances when you talk about “homocytinuria”, explain what is actually found in urine in the way of homocysteine derivatives, and explain how you converted to tHcy (although that may or may not be appropriate in this situation, given that there is probably little or no free homocysteine in urine).

(DV: Yes, on these pages I will change the line above the tables to "Cystathionine beta Synthase deficiency by severe homozygous or compound heterozygous mutations"

Yes, I will reproduce the Bonham (1987) and Wiley (1989) algorithms on p27 at the bottom of Table 1, to also appear under the section heading on p 54)

HM: More importantly, I do agree with the need to recalculate treatment outcomes in the studies such as those in the Yap et al paper. However, I have not had time to go over the details you present on this matter. Again, I may have missed it, but another concern I have is that the time-to-event curves for B6-responders presented by Mudd et al 1985 may have been severely affected by ascertainment bias (you must have seen the paper by Skovby et al (Mol. Genet. Metb 101, 172-177, 2010). If the tentative conclusion in that paper holds up, the risk

for untreated B6-responders in Mudd et al 1985 probably was severely overestimated. You may choose to at least mention this possibility.

(DV: I agree, and I did address this on p 24-25 where I noted that the relative risk of .09 given by Yap et al. was actually closer to 0.2 when determined using only the actual portions of your (1985) survival curves that are applicable to the cases, this being a two-fold exaggeration of efficacy, and that consideration of the ascertainment bias you note would indicate probably nearly a three-fold exaggeration of the real efficacy in total.)

HM: Hope this helps. Use it as you wish, but please do not quote me in a way that suggests I have gone over your draft in detail. I may or may not find time to read more carefully those portions on which I might have further comments (e.g. chapter3), but my schedule is too crowded to offer even a vague deadline to try to do so.

(DV: Thank you very much Prof. Mudd, as noted from the outset there should be virtually no chance of such misrepresentation because all of your comments here, and my responses to them, will be reproduced verbatim in their entirety in the last section/ chapter of the published book.

Any further commentary would be very welcome, and as I am self-publishing via Amazon's CreateSpace (Print on Demand), even if you comment after the initial publication, which is unlikely to be sooner than two weeks from now, I will be able to modify the book as printable in the future.)

Harvey Mudd

Response from Professor Bridget Wilcken:

BW: Dear David, I have just been re-reading your paper.

While I think there are excellent points, there are also aspects that you haven't taken into account. Firstly, it is evident that the treatments, various, do result in benefit with regards to occurrence thrombo-embolism (and maybe that should in fact be vascular disease in general),

(DV: Yes, but unfortunately, as I noted in my work under discussion here, and as you have not noted in your comments here, the thrombo-embolism is very likely to still be substantially above the level of the general population (except in the Dublin cases where there isn't any at all…..), and therefore it is desirable to reduce it still further if possible, for example by some sensible, safe, cost-effective method(s), perhaps such as I have suggested…..)

BW: and that we do not really know what aspects of the treatments are the most important.

(DV: Indeed "we do not know what aspects of the treatments are most important"– that is why I carried out my study under discussion here – to further elucidate what aspects of treatment may be not necessarily most important (which is not quite the point) but be important enough to be done (which is more the point).)

BW: It is clear that the treatments used all resulted in lowering of homocysteine levels, but that the great majority of patients continued to have very significant elevations.

(DV: And therefore will have very significant decreases in cysteine, unless receiving it in supplements, particularly if they are receiving folate and/or vitaminB12 and/or betaine (which they are….) as explained again here below in response to your response here.)

BW: However, it is not at all reasonable to compare Dublin (and maybe Manchester also) with the other centres, as almost all the patients from Dublin and most from Manchester were diagnosed by screening (newborn or family studies) and started on treatment in the first few months. Thus the homocysteine levels that group obtained would have been extremely well-controlled in childhood.

(DV: I"ll begin here by agreeing with you that I haven"t dealt sufficiently with the differences between Dublin (and less so Manchester) and the other centers regards the former centers' patients being on treatment in early childhood whereas the latter centers' patients were not. I fully acknowledge this oversight. And I have now included the following points in the discussion in my work under discussion here.
However, comparing the centers with each other is quite reasonable if it is done reasonably, adjusting properly for relevant factors where known and possible, and properly noting the caveats including known factors not adjustable for or adjusted for, and mentioning as far as possible the possibility and probability of factors that might be unknown.
Firstly, the homocysteine levels of the Dublin cases are nonetheless in the middle of the range of the five centers (tHcy approximately 110 micromoles per liter versus approximately 80-130), though it might well be the case that the B6-non-responsive cases in the other centers have higher tHcy, and the B6-responsive cases lower tHcy, than the combined averages given.
Now, if early treatment confers some advantage other than later adherence to homocysteine- lowering treatment, it is very likely to be due to differences in the body"s more permanent structures, rather than to differences in less permanent things like platelets and biochemicals in solution.
For example the protein fiber scaffolding of tissues including the walls of arteries and veins, in particular the protein fibrillin with its unusually high cysteine content and cysteine-cysteine double bonds.
Furthermore, the Irish (Dublin) cases are nearly all of the more severe vitamin B6-unresponsive type whereas all the other centers are much closer (roughly 1:1 to 1:2) to being evenly comprised of B6-unresponsive and B6-responsive (which fact I neglected to point out in discussion (although the facts are clear in the table), and thank you for inadvertently causing to me to have to look again to respond to your comments, and I have now added that point to the discussion).
Such cases would nearly all be detected clinically within the first five years of life. It is possible that clinical detection would drive a higher compliance with treatment, due to non-trivial disease manifestations being already experienced by the patient or members of their family, whereas this is less so for those ascertained via family studies, and even less so for those ascertained by population screening.
Compliance is of course a function of the experienced burden of the treatment, which might be reasonably said to be greater insofar as it included the unpalatable cysteine supplementation (though this should be

able to be given as a single amino acid in a capsule(s) rather than as a more voluminous mixture of amino acids, relying on food for the other amino acids).
However compliance is also a function of patient-doctor contact type and frequency. It should be borne in mind that Dublin has absolutely no events of thrombosis, as yet.
Note that there is no ascertainment bias unreasonably against the other four centers in comparison, because the Dublin cases are absolutely more severe – if anything the ascertainment bias would be unreasonably in favour of the other four centers in comparison, even if this is in the reverse direction to most ascertainment bias noted in the field of epidemiology.
The point is that when, as my numerical data analysis does, the case severity is adjusted for, which is a substantial improvement over all prior such studies (which you do not acknowledge and seem not to understand), Dublin does better.
I do not claim that this proves that Dublin‟s treatment practice is better, I claim only that it suggests it.
And consideration also of other facts as provided in my work under discussion here provides a reasonable theoretical support for cysteine supplementation being important.)

BW: From my long experience of treating large numbers of phenylketonuria subjects, who also need a low-protein diet with a (different) amino acid supplement, I can say that it is usually not possible to get them to comply fully unless treatment is started in infancy. The taste of the supplement is simply not acceptable to many unless they have become accustomed in infancy.

(DV: I am well aware of the taste factor, and thought that this was sufficiently well implied to the expected readership when I raised compliance with treatment as an issue, and explicitly suggested that compliance will be better when treatment is implemented early and doctor-patient contact type and schedule is optimized.
However, again, it seems to me that cysteine supplementation should be able to be given as a single amino acid in a capsule(s) rather than as a more voluminous mixture of amino acids, relying on food for the other amino acids, sufficiently increased compliance being the justification.)

BW: I don't think therefore that it is valid to make a sudden, evidence-free leap, and say that cysteine supplementation is likely to be a major factor in better results. It could be so and it seems quite reasonable that more cysteine could be needed, but the better results obtained by Dublin are not sound evidence for this. They are much more likely to be due to earlier treatment

(DV: My suggestion is not a "leap", and it is most certainly not "evidence-free".
More the point, you are one of the experts that have unfortunately overlooked the significance of low cysteine as the other half (versus high homocysteine) of one of the main, if not the main problem(s).
Note that the area of structural tissue involved in the ectopia lensis (eye lense displacement) of CBS--homocystinuria has a very high (almost singularly so, compared to other body proteins) content of cysteine in its proteins, and cysteine-cysteine double bonds in its structure. To copy verbatim from my work being discussed here by way of citing references contributing evidence "In accord with the enzyme defect in CBS it has been shown that requirement for cysteine is increased in CBS--: Perry et al (1968), Komrower et al (1966), Perry et al (1966), Brenton et al (1966), Laster et al (1965). …… ……. ……. Majors and Pyeritz (2000) report their investigations following on speculation by themselves and others that in CBS-- the altered plasma concentrations of homocysteine and/or cysteine may hinder the synthesis, deposition, or both, of fibrillin-1. They report that when arterial smooth muscle cells were cultured under conditions of cysteine deficiency fibrillin-1 deposition into the extracellular matrix was greatly diminished (and restored with addition of

cysteine), but that excessive homocysteine in contrast had little if any effect on fibrillin-1 deposition. Furthermore, in the reference book Homocysteine in Health and Disease (Carmel and Jacobsen, editors (2001) p238) Kraus and Victor cite Mudd and Levy (1978) in the universally accepted as authoritative The Metabolic Basis of Inherited Disease to the effect that "Dietary manipulation of methionine and cysteine intake is an option for treating patients who are partially or fully nonresponsive to vitamin B6. ……. For patients with no residual CBS activity, cysteine must be provided……."
Now, if treatment of CBS homocystinuria (in particular, but not only, the partially vitaminB6-responsive cases with vitamin B12 and/or folate and/or betaine causes a further reduction of flow of homocysteine through the transulfuration pathway and therefore further reduction of cysteine production, because the homocysteine escapes through the remethylation pathway instead, considered with all the evidence available, including but not limited to my data analysis (which you have not well if at all understood) in the work under discussion here, it is hardly a "leap" to suggest that failure to provide or take supplemental cysteine is likely to cause problems in later life.
I acknowledge that you have other types of cases than CBS homocystinuria to deal with, whereas I have made the time to specialize in studying homocysteine for a few years, but I suggest that you, and more the point your patients, would benefit from yourself studying the situation further, regards aspects that I have raised, and changing your practice accordingly, unpalatable as that may be coming from someone outside your network. Furthermore, your statement "They are much more likely to be due to earlier treatment" is much more of an "evidence-free leap" than my suggestions, offering as it does no supporting evidence, and certainly my extensive reviewing of the literature (see also the 100-page tabulation of extracted data and word descriptions in the file on the Multifactor Health website {here above, now}) has not revealed any evidence in support of that statement. More likely you resent an outsider correcting a deficiency in your practice, and respond in a way very devoid of the objectivity and logic that are at least the cornerstones, if not much more as well, of scientific rigour.)

BW: Now that we are screening for homocystinuria, there is accumulating evidence about the value of early instigation of treatment – not that this was not extremely likely, but now there is some slowly gathering data. There is some other evidence for the value of vitamin C (Bonham et al – cant look it up at present, I fear)

(DV: Other than in Pullin, Bonham and McDowell (2002) as already cited in my work being discussed here, it must be very recent, and I would be pleased to add it to the literature review if available)

BW: , and most of our older patients are on this

(DV: Why only the older patients?).

BW: I am writing this without the benefit of referring to our combined paper, as, unkindly, I am not able to access the internet due to some server glitch. This will have changed perforce, if I can send this to you, so I may add something at the end. But I thought that the method of computing the expected number of thrombo-embolic events was a simple method of summing the numerical risk for each patient derived from the Mudd graph. Then it is not necessary to know the average age at treatment. Eg, from Mudd's graph one can see that the risk for patient "A" aged 20 years and B6 non-responsive, if untreated would be about 0.26 (I cant see the graph very clearly), for patient "B" B6 responsive and aged 32 would be about 0.51, for patient "C" B-6 non-responsive and aged 6y would be c. 0.04, and so forth.

(DV: No, one may not reasonably claim a treatment success for time periods that one has not been applying

the treatment – this had the effect in the Yap et al (2001) paper of inflating the actual treatment success from a risk rate of approximately 0.2 (by my calculations using only the (average) periods the patient groups were actually treated for) to one of .09, which I noted in my work (also noting that I would not belabor the point, as I was then inclined to be a little less combative than I presently am inclined) which is not a trivial difference (It is a claim to have been approximately twice as successful as one could reasonably claim, and probably closer to three times as successful as one could reasonably claim, for four non-Dublin groups, considering the ascertainment bias towards more severe cases of the vitaminB6-responsive cases in the Mudd (1985) study used for comparison to derive the risk ratio in Yap et al (2001)).
Indeed, had I had the individual data, I would have used them instead of the averages, but the small gain in precision thereby achieved would not much change the outcome of my numerical analysis, and it certainly would not negate the fact of my analysis being far more reasonable than that of Yap et al (2001) due to my use of the appropriate time period (the period during which treatment actually occurred).
Furthermore, Yap et al (2001) did not at all sufficiently detail their numerical analysis methods, leaving it to readers to have to trial various possible methods in order to determine what they had in fact done – doubtless very few readers if any other than myself actually did so, although some may well have realized what shortcomings the lack of given detail could imply.)

BW: Unfortunately I found your statistical analysis difficult to follow, but I am not expert in that area. However, I'm not sure it was necessary in the first place.

(DV: The statistical analysis is indeed more difficult to follow than much of what appears in the literature. I also would not call myself an expert statistician, or even rank myself with the very competent. I am, however, better qualified and more competent as an epidemiologist than the average medical doctor, and also than the average journal article author with a medical degree, judging from the shortcomings and mistakes that I repeatedly or very often find in the hundreds of journal articles that I review. I hope you will understand, with further consideration, the appropriateness of my statistical analysis here. If not, there are various courses that you can take to improve competence in that field, should your time commitments permit and you incline to.)

BW: From a publication point of view your paper would need very substantial revision. It is much too long for most journals, and I would advise aiming for a text of c. 3,000 words, certainly less than 4,000

(DV: To revise it back to this length would remove so much material as to reduce the quality of the work unacceptably, which should be somewhat obvious – if it is too long for a standard journal article, then so be it, for the moment.) .

BW: It is also not very focused

(DV: It is quite appropriately focused, on optimizing the treatment of cystathionine beta synthase deficiency homocystinuria) .

BW: I think the discussion section (which is not a discussion at all of what you have analysed)

(DV: It is a discussion, including of what I have analysed, of what is potentially relevant to optimizing the treatment of cystathionine beta synthase deficiency homocystinuria.)

BW: is interesting, and it might be that your work could become publishable if it was mainly focused on this

– a review of possible treatment modalities. Homocystinuria is relatively rare and the clinical outcomes are very long-term

(DV: Ultimately all clinical outcomes should include old age, and this is hardly unique to homocystinuria, in which outcomes addressed in formal analysis to date have only included life periods up to and including middle age, not old age, which is an explicitly stated reason for seeking to further optimize treatment.)

BW: , which is why we have so little reliable information about ideal treatment aims. (One thing of note, not obviously related to thrombo-embolism, is the much better intellectual outcome with early treatment. Quite a proportion of B6 non-responsive patients clinically diagnosed have intellectual handical to some degree, whereas it seems likely from screening data that this does not occur at all with early treatment.)

(DV: This has been quite well noted for many years now.)

BW: I'm sorry to sound negative overall. I am not sure I can be of much more help with this, and certainly ought not to be an author.

Kind regards,

Bridget

Professor Bridget Wilcken AM, MD, FRACP, FRCPA(hon)
NSW Biochemical Genetics and Newborn Screening Service
The Children's Hospital at Westmead, Sydney, Australia
(Contact details removed by Vance as a courtesy, they will be readily available elsewhere)